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LATENT PERIPHERAL ACTIONS	• FUNCTIONS INHIBITED BY EPINEPHRINE: INTESTINE ISOLATED • EPINEPHRINE VASODILATATION • VASOCONSTRICTION AND PRESSOR ACTION IN MAN CAUSED BY NICOTINE, VALSALVA MANEUVER, COLD, HYPERVENTILATION • STRIATED MUSCLE • METABOLIC EFFECTS OF EPINEPHRINE, I.E., INCREASE OF BMR, EPINEPHRINE HYPERGLYCEMIA • RENAL CORTEX ISCHEMIA FOLLOWING EPINEPHRINE, ASPHYXIA, ELECTRIC STIMULATION OF THE SPLANCHNIC NERVES, CRUSH SYNDROME	ADRENERGIC BLOCKADE NO BLOCKADE PREVENTION OR INHIBITION RELAXATION (LISSIVE) INHIBITION PREVENTION

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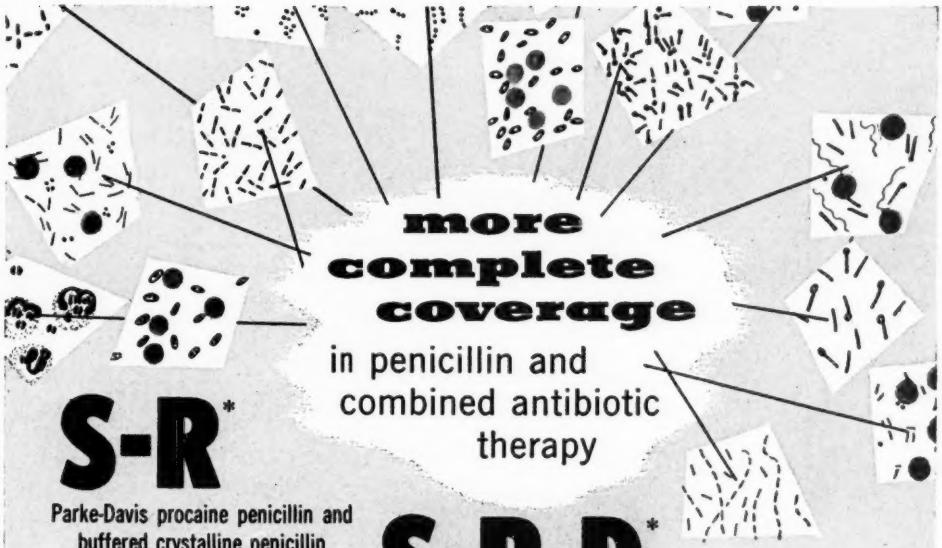

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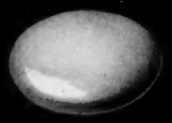

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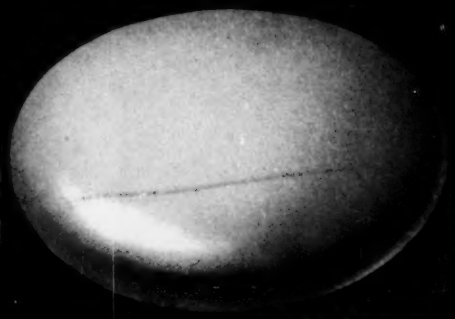

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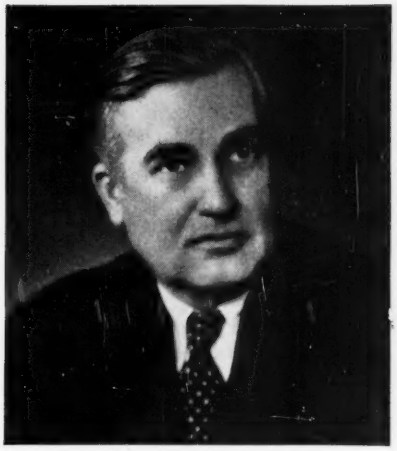

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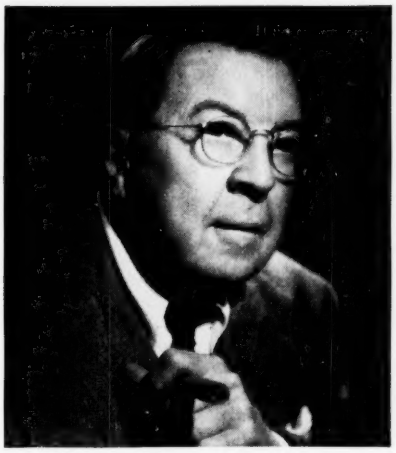

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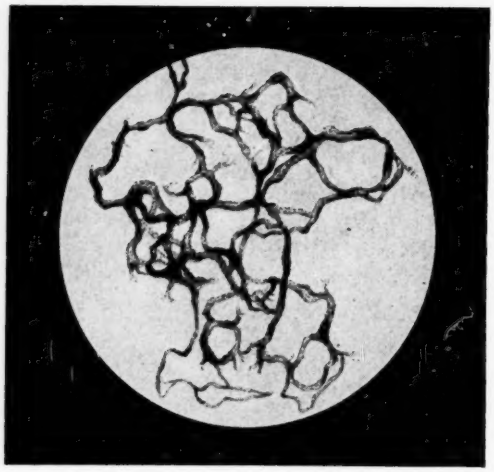

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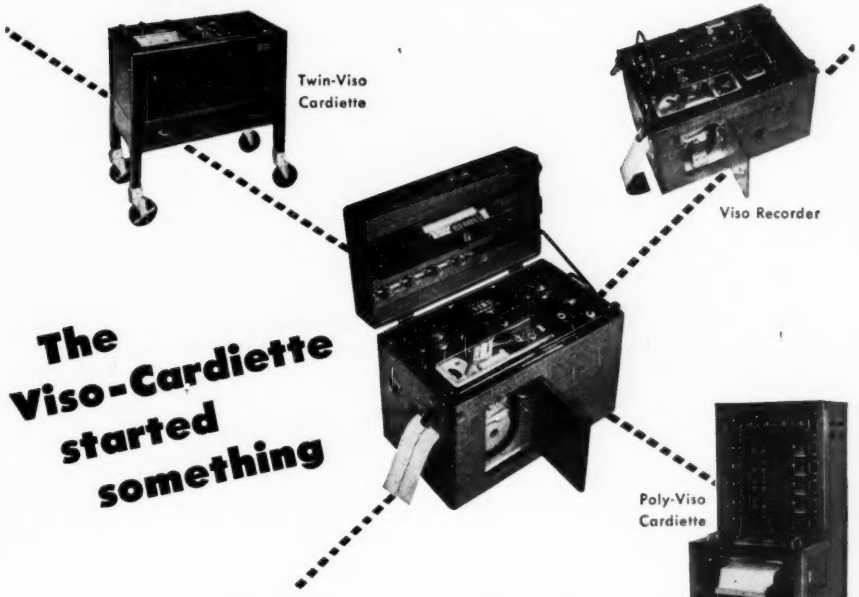

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THE EFFECT OF BREATHING 100% OXYGEN ON THE PULMONARY ARTERIAL PRESSURE IN PATIENTS WITH PULMONARY TUBERCULOSIS AND MITRAL STENOSIS [1]

By SIDNEY H. DRESSLER,[2] N. BALFOUR SLONIM,[3] OSCAR J. BALCHUM,[4] GERALD J. BRONFIN, AND ABE RAVIN

(From the Cardio-Pulmonary Laboratory of the National Jewish Hospital at Denver and the Department of Medicine of the University of Colorado Medical Center, Denver, Colo.)

(Submitted for publication August 10, 1951; accepted May 14, 1952)

The breathing of anoxic mixtures causes an increase in the pulmonary arterial pressure of animals (1–4) and man (5–7). This effect has been attributed to a local action of the anoxic mixture on the blood vessel walls resulting in a constriction of the pulmonary arterioles and pre-capillaries and an increase in pulmonary arterial pressure. Vagotomy and stellate ganglionectomy do not change the vascular response to the breathing of anoxic mixtures in the cat (1).

The effect of breathing 100% oxygen on the pulmonary arterial pressure has been investigated in animals (1, 3, 4, 8) but not adequately in man. Euler and Liljestrand (1) found that pure oxygen caused a fall in the pulmonary arterial pressure of cats which did not appear to these investigators to be the result of decreased blood flow, but rather to a dilatation of the pulmonary vasculature. The response to oxygen was not affected by vagotomy and stellate ganglionectomy. Ergotamine, yohimbine, and atropine (9) did not alter the decrease of pulmonary arterial pressure which resulted from oxygen breathing.

This paper reports observations on the effect of breathing 100% oxygen on the pulmonary arterial pressure of patients with pulmonary tuberculosis and mitral stenosis.

METHODS

Two groups of patients were studied. One group consisted of 21 individuals with pulmonary tuberculosis who were studied 27 times. These were all patients with moderately and far advanced pulmonary tuberculosis (NTA criteria) whose disease was chiefly unilateral. The second group consisted of 17 individuals with rheumatic heart disease manifested by mitral stenosis who were studied 25 times.

The morning of the catheterization, the patients received .09 gram of nembutal orally and arrived at the laboratory one hour later in the fasting state. Pulmonary artery catheterization was performed in the usual fashion. A blood sample was taken from the brachial artery for determination of the control oxygen saturation, and pressure-pulse records of the pulmonary arterial pressure were made. When the control pressure level had been established the patient was given 100% oxygen [5] by BLB mask for a period of four minutes. In 12 instances pulmonary arterial pressure tracings were made continuously during and for ten to 12 minutes after oxygen administration, but in the majority a record was made only when oxygen had been given for three minutes. A sample of brachial arterial blood was withdrawn at the fourth minute of oxygen administration for determination of oxygen saturation.

Arterial blood samples were analyzed for oxygen content and capacity by the method of Van Slyke and Neill (10), and more recently by the Scholander-Roughton syringe method (11). The pulmonary arterial pressure was recorded with a Hathaway Blood Pressure Control Unit and Oscillograph [6] and in many instances was simultaneously recorded with a Sanborn Electromanometer connected to the Hathaway Oscillograph. Recordings were calibrated with a mercury manometer after each tracing. The zero point for all pressures was 5 cm. posterior to the sternal angle of Louis with the patient supine. Mean pressures were obtained by planimetric integration of the pressure-pulse contours when the Hathaway Blood Pressure Recording System was used and by electrical integration when the Sanborn Electromanometer was used.

The respiratory pattern as evidenced by rhythmic baseline fluctuation was present in all pressure tracings, except in those of patients with mitral stenosis who had auricular fibrillation. Representative expiratory and inspiratory

[1] Aided by a grant from the Life Insurance Medical Research Fund.

[2] Medical Director of the National Jewish Hospital at Denver.

[3] Public Health Service Postdoctorate Research Fellow of the National Heart Institute.

[4] Fellow of the National Research Council.

[5] The mean annual barometric pressure in Denver is 628 mm. Hg.

[6] Type SYBP-1 Blood Pressure Recording System.

pressure-pulse contours were taken for each measurement, and the average systolic, diastolic and mean values recorded. Only mean pressures were used in the statistical analyses. Measurements of all pressure tracings were rounded to the nearest integer, and five-tenths was rounded to the nearest even integer.

RESULTS

1) *Pulmonary arterial pressure*

Of a total of 52 studies made on patients with pulmonary tuberculosis and mitral stenosis, 43 showed a decrease in pulmonary arterial pressure during oxygen breathing, three showed no change, and six showed slight increases (Tables I, II and III). The decreases ranged from 1 to 18 mm. Hg systolic, from 1 to 12 mm. Hg diastolic, and from 1 to 13 mm. Hg mean.

Out of 27 studies in patients with pulmonary tuberculosis, 22 showed a decrease of pulmonary arterial pressure with oxygen, two showed no change, and three showed slight increases. Where a decrease occurred, the average drop in systolic pressure was 4 mm. Hg (17% of the control level), the average drop in diastolic pressure was 3 mm. Hg (33% of the control level), and the average drop in the mean pressure was 3 mm. Hg (19% of the control level). In the three instances where an increase was noted, the rise averaged 2 mm. Hg systolic, 2 mm. Hg diastolic, and 1 mm. Hg mean.

Out of 25 studies in patients with mitral stenosis, 21 showed a decrease in pulmonary arterial pressure with oxygen, one showed no change, and three showed slight increases. Where a decrease occurred, the average drop in systolic pressure was 7 mm. Hg (16% of the control level), the average drop in diastolic pressure was 5 mm. Hg (21% of the control level), and the average drop in the mean pressure was 4 mm. Hg (13% of the control level). In the three instances where an increase was noted, the rise averaged 2 mm. Hg systolic, 1 mm. Hg diastolic, and 1 mm. Hg mean.

TABLE I

The effect of breathing 100% oxygen on pulmonary arterial pressure, pulse rate, and arterial saturation in patients with pulmonary tuberculosis

Patient	Pulmonary arterial pressure*									Pulse rate			Arterial saturation		
	Control			Oxygen			Change			Control	Oxygen	Change	Control	Oxygen	Change
	S	*D*	*M*	*S*	*D*	*M*	*S*	*D*	*M*						
AFD	23	10	16	16	6	10	− 7	−4	−6	95	80	−15	93	98	+ 5
KLW	28	12	20	23	10	16	− 5	−2	−4	115	95	−20	94	100	+ 6
BJP	24	7	18	23	8	16	− 1	+1	−2	95	90	− 5	91	99	+ 8
BJP	21	8	15	17	7	13	− 4	−1	−2	100	90	−10	91	92	+ 1
LMW	23	7	15	26	9	16	+ 3	+2	+1	80	80	0	95	99	+ 4
LMW	26	8	16	28	8	17	+ 2	0	+1	85	80	− 5	88	98	+10
DCL	15	4	10	12	4	9	− 3	0	−1	75	75	0	96	99	+ 3
DCL	16	6	12	14	8	10	− 2	+2	−2	85	72	−13	88	94	+ 6
RRS	12	8	10	12	4	8	0	−4	−2	92	90	− 2	92	97	+ 5
MV	22	6	14	15	2	11	− 7	−4	−3	85	85	0	93		
RL	16	7	10	12	4	8	− 4	−3	−2	100	100	0	90	100	+10
RL	24	3	15	18	2	12	− 6	−1	−3	90	85	− 5	90	98	+ 8
TR	24	8	16	18	4	11	− 6	−4	−5	105	85	−20	93	98	+ 5
TR	26	8	15	22	6	15	− 4	−2	0	85	75	−10	93	99	+ 6
LS	28	13	20	26	8	18	− 2	−5	−2	95	80	−15	90	98	+ 8
FG	30	7	16	24	6	14	− 6	−1	−2	115	105	−10	91	96	+ 5
AC	20	6	14	21	6	14	+ 1	0	0	80	70	−10	90	97	+ 7
FJG	26	9	18	22	8	16	− 4	−1	−2	95	88	− 7	90	98	+ 8
EV	22	10	16	20	8	12	− 2	−2	−4	100	100	0	85	96	+11
LEV	24	9	16	20	6	13	− 4	−3	−3	90	100	+10	94	97	+ 3
AG	22	14	18	18	8	12	− 4	−6	−6	85	75	−10	94	98	+ 4
FVG	39	24	30	28	16	21	−11	−8	−9	95	92	− 3	84	95	+11
VFP	25	6	14	22	4	12	− 3	−2	−2	82	72	−10	87	95	+ 8
VFP	20	6	13	20	6	12	0	0	−1	97	95	− 2	92	98	+ 6
GAR	20	6	12	21	4	10	+ 1	−2	−2	80	72	− 8	90	98	+ 8
JFA	14	4	8	12	4	9	− 2	0	+1	107	100	− 7	92	94	+ 2
IN	30	10	17	26	8	14	− 4	−2	−3	82	72	−10	88	98	+10
Average	23	8	15	20	6	13	− 3	−2	−2	92	85	− 7	91	97	+ 6

* S = systolic, D = diastolic, and M = mean.

TABLE II

The effect of breathing 100% oxygen on the pulmonary arterial pressure, pulse rate, and arterial saturation in patients with mitral stenosis

Patient	Pulmonary arterial pressure*									Pulse rate			Arterial saturation		
	Control			Oxygen			Change			Control	Oxygen	Change	Control	Oxygen	Change
	S	*D*	*M*	*S*	*D*	*M*	*S*	*D*	*M*						
LHS†	26	10	17	28	12	19	+ 2	+ 2	+ 2	65	65	0	87	99	+12
LHS†	38	16	26	34	15	25	− 4	− 1	− 1	55	55	0	91	98	+ 7
RJD	41	22	28	40	20	26	− 1	− 2	− 2	60	55	− 5	90	98	+ 8
RJD	34	12	21	33	12	20	− 1	0	− 1	63	57	− 6	93	99	+ 6
HMW	33	15	21	30	15	19	− 3	0	− 2	58	58	0	94	98	+ 4
CFW	58	28	40	44	16	27	−14	−12	−13	75	50	−25	95	100	+ 5
CFW	49	34	38	34	24	30	−15	−10	− 8	100	85	−15	86	96	+10
HJS	72	32	52	62	30	46	−10	− 2	− 6	82	80	− 2	90	98	+ 8
TB‡	32	20	24	29	14	22	− 3	− 6	− 2	90	82	− 8	90	96	+ 6
TB‡	44	22	31	38	15	26	− 6	− 7	− 5	75	64	−11	89	97	+ 8
ND	64	24	33			26			− 7	87	80	− 7	91	97	+ 6
ERH‡	21	6	15	22	6	14	+ 1	0	− 1	85	90	+ 5	94	96	+ 2
ERH‡	41	13	26	35	12	25	− 6	− 1	− 1	82	80	− 2	90	94	+ 4
BLP‡	40	18	29	35	16	27	− 5	− 2	− 2	90	110	+20	94	99	+ 5
CLR‡	52	26	34	48	20	32	− 4	− 6	− 2	98	96	− 2	89	97	+ 8
CLR‡	48	22	33	41	22	29	− 7	0	− 4	66	62	− 4	88	92	+ 4
GER‡	54	32	41	36	22	29	−18	−10	−12	90	82	− 8	95	100	+ 5
GER‡	31	13	22	33	14	23	+ 2	+ 1	+ 1	105	95	−10	94	98	+ 4
MTD‡	35	16	24	32	16	23	− 3	0	− 1	85	80	− 5	88	96	+ 8
MTD‡	42	18	25	27	8	17	−15	−10	− 8	64	60	− 4	93	93	0
EMB	40	13	26	35	14	24	− 5	+ 1	− 2	75	65	−10	94	98	+ 4
EBW	29	10	17	26	10	18	− 3	0	+ 1	65	70	+ 5	88	97	+ 9
BLM‡	39	12	24	36	12	24	− 3	0	0	80	66	−14	89	94	+ 5
FCD‡	53	24	37	48	22	32	− 5	− 2	− 5	70	57	−13	92	94	+ 2
EM‡	68	29	36	62	28	34	− 6	− 1	− 2	65	60	− 5	93	100	+ 7
Average	43	19	29	37	16	26	− 6	− 3	− 3	77	72	− 5	91	97	+ 6

* S = systolic, D = diastolic, and M = mean. † Auricular flutter. ‡ Auricular fibrillation.

For the pulmonary tuberculosis group the standard error of the mean of the differences between the mean pulmonary arterial pressures before and during oxygen breathing was determined from standard statistical formulas.[7] The mean pair-difference divided by the standard error gives a *t* value of 4.5. This shows a highly significant difference between the control pulmonary arterial pressure and the pressure during oxygen breathing, since this *t* value corresponds to a P of less than .00006. For the mitral stenosis group the mean pair-difference divided by the standard error gives a *t* value of 3.9 which corresponds to a P of less than .0001.

The results obtained in 12 cases where the pulmonary arterial pressure was recorded continuously during and after oxygen breathing are shown in Figures 1 and 2. In Figure 1 the cases are grouped according to diagnosis and in Figure 2 according to control pressure level. The pressure decrease is quite apparent at one minute and almost complete at two minutes, with only slight change during the third and fourth minutes. Two minutes after the cessation of oxygen breathing the pressure has shown very little rise, but at six and ten minutes there has been a partial return to the control level.

2) Level of pulmonary arterial pressure and degree of change

There was a definite association between the control mean pulmonary arterial pressure and the degree of its fall during oxygen administration. The regression line of the change in mean pressure on the control pressure for the pulmonary tuberculosis group is given by the equation $Y = -.35X + 3.0$, while that for the mitral stenosis group is $Y = -.31X + 5.7$. The regression coefficients are very similar for the two groups of patients. Of the patients who showed a decrease in pulmonary arterial pressure with oxygen, the average fall of the 11 with the highest control pres-

[7] Fisher, R. A., Statistical Methods for Research Workers. Oliver & Boyd, Edinburgh, 1950, 11th ed.

TABLE III

The effect of breathing 100% oxygen on pulmonary arterial pressure and pulse rate in patients with pulmonary tuberculosis and mitral stenosis

			Pulmonary tuberculosis			Mitral stenosis			Total		
			Decrease	Increase	No change	Decrease	Increase	No change	Decrease	Increase	No change
Number of instances			22	3	2	21	3	1	43	6	3
Pulmonary arterial pressure*	Change: *Average and Range*	S	−4 (17%) −1 to −11	+2 (8%) +1 to +3		−7 (16%) −1 to −18	+2 (6%) +1 to +2		−5 (17%) −1 to −18	+2 (7%) +1 to +3	
		D	−3 (33%) −1 to −8	+2 (25%) +1 to +2		−5 (21%) −1 to −12	+1 (11%) +1 to +2		−4 (27%) −1 to −12	+2 (18%) +1 to +2	
		M	−3 (19%) −1 to −9	+1 (8%) +1		−4 (13%) −1 to −13	+1 (7%) +1 to +2		−3 (16%) −1 to −13	+1 (8%) +1 to +2	
Pulse rate	Number of instances		18	1	5	17	3	3	35	4	8
	Average change		−10	+10		−9	+10		−10	+10	

* S = systolic, D = diastolic, and M = mean.

sures was 18%, and the average fall of the 11 with the lowest control pressures was 15%.

The actual drop in pressure in the patients with pulmonary tuberculosis was less than in the mitral stenosis patients, possibly because the control pulmonary arterial pressure was lower. The change in patients with a normal pressure was often of a small degree. However, of the 14 instances where the control pressure was normal (15 mm. Hg or less), ten showed a decrease in pressure, two showed no change and two showed increases of 1 mm. Hg each. Calculations in these 14 instances, however, revealed a statistically significant decrease of the mean pulmonary arterial pressures during oxygen breathing. The t value for this is 3.67 which corresponds to a P of less than .01. Thus the observed decreases would occur less often than once in a hundred times by chance alone.

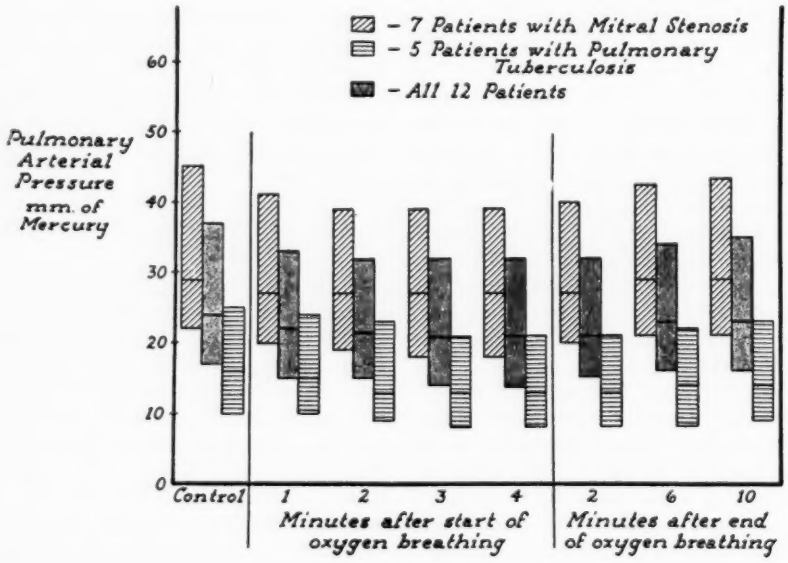

FIG. 1

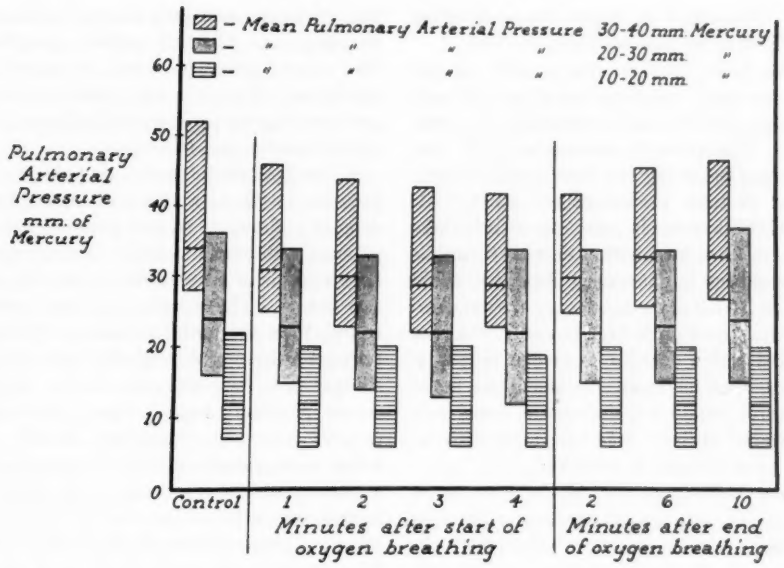

FIG. 2

Although these patients had a normal pulmonary arterial pressure, it should be noted that they had in general rather extensive pulmonary disease.

3) Pulse rate

The pulse rate decreased together with the mean pulmonary arterial pressure in 34 instances (Tables I, II and III). However, in three instances where the pulse rate increased, the mean pulmonary arterial pressure decreased. In six instances where the pulse rate showed no change, there were decreases in the mean pulmonary arterial pressure. In three instances pulse rate decreases were associated with no change in the mean pulmonary arterial pressure.

4) Arterial saturation

A study of control arterial oxygen saturations (range from 84% to 96%) and changes in the mean pulmonary arterial pressure resulting from oxygen breathing failed to reveal an appreciable correlation. However, the range of arterial oxygen saturations was not great. It is interesting to note that the patient with the highest mean pulmonary arterial pressure in the pulmonary tuberculosis group had the lowest control arterial saturation and showed the greatest drop in pressure with oxygen.

DISCUSSION

The data presented indicate that individuals with pulmonary tuberculosis and mitral stenosis at the barometric pressure in Denver show a decrease in pulmonary arterial pressure during oxygen breathing. Such a decrease could be due to: *1*) a reduction in the volume of blood flow through the pulmonary vascular bed, *2*) a diminution of pulmonary vascular resistance, or *3*) a fall of the left atrial and pulmonary venous pressure. A decrease in cardiac output during oxygen breathing in man has been noted by several investigators using the ballistocardiograph (12–14). This decrease has been estimated to be from 10% to 20% and is reflected initially by a decrease in pulse rate. It has been reported that during the first five minutes of oxygen breathing, approximately 94% of the decrease in cardiac output is reflected in a decrease of pulse rate and the remainder is reflected by a decrease of stroke output (12). Although there is some question regarding the validity of absolute values for cardiac output as determined by the ballistocardiograph, it seems

likely that the changes in output observed during oxygen breathing by this method are valid.

That the pulse rate decreases with oxygen breathing has been frequently noted in both animals and man (15–18) and is evident in the present study. This prompt decrease in pulse rate during oxygen breathing has been attributed to a moderation of tonic chemoreceptor activity (1, 14, 19). If the decrease in pulse rate seen in these patients were associated with a decreased cardiac output, the change in pulmonary blood flow could be a factor in the fall of the pulmonary arterial pressure. A decrease of pulse rate, however, need not be associated with a decrease of cardiac output, if the stroke output increases. It should be noted that in several instances a decrease in mean pulmonary arterial pressure was associated with no change or even increase in pulse rate.

Euler and Liljestrand did not believe that a change in cardiac output could account for the drop in pulmonary arterial pressure which they obtained, since the change in pressure was greater than that obtained by marked exercise. It has also been shown that in normal individuals exercise of fairly marked degree has little effect on pulmonary arterial pressure (20). Thus it is unlikely that a small change in cardiac output would be an important factor in decreasing the pulmonary arterial pressure. However, until the cardiac output has been measured by more exact methods in both animals and man, the part played by changes in pulmonary blood flow cannot be fully evaluated. In studies on the effect of acute anoxia on the human pulmonary circulation, the cardiac output has not been found to show a definite change (5, 7), and certainly has not changed enough to explain the pulmonary arterial pressure rise.

Because Euler and Liljestrand did not believe that changes in cardiac output could account for the drop in pressure, and because they and others found that the drop occurred in the absence of the nerve supply, they postulated that the fall in pressure was due to a direct action of oxygen on the pulmonary vasculature producing vasodilatation. Our experience with one patient may indicate that in man, as well as in animals, the drop in pulmonary arterial pressure is not dependent upon an intact nerve supply. This patient had bronchial asthma (not included in tables or figures), and had previously undergone bilateral pulmonary hilar stripping and bilateral stellate ganglionectomy. The control pressures were 26 systolic, ten diastolic, and 18 mean. After three minutes of oxygen breathing the pressure had fallen to 19 systolic, eight diastolic, and 14 mean.

A change in the left atrial and pulmonary venous pressure might be a factor of importance in the drop in pulmonary arterial pressure noted in the patients with mitral stenosis. Pulmonary arterial hypertension in these patients may depend upon two factors. The elevated left atrial pressure resulting from the mitral stenosis is transmitted in retrograde fashion through the pulmonary vascular system to the pulmonary artery. Early in the course of mitral stenosis this is the only factor causing increased pulmonary arterial pressure. Later, many patients develop increased pulmonary arteriolar resistance which is associated with a further rise in pulmonary arterial pressure. This increased resistance may be the result of pathological changes in the small vessels or it may be due to vasoconstriction. Oxygen breathing might decrease the pulmonary arterial pressure in patients with mitral stenosis by reducing the pressure in the left atrium or by a vasodilatory effect on the pulmonary vessels. The decreased cardiac rate induced by oxygen breathing permits better left ventricular filling and thus increases the stroke output of that chamber, resulting in turn in a decrease of the left atrial, pulmonary capillary and pulmonary arterial blood volumes and pressures. The relative importance of a decrease in left atrial pressure and the dilatation of the pulmonary vessels might be evaluated by determination of the effect of oxygen breathing on the pulmonary "capillary" pressure. However, it must first be proved that the pressure obtained through a catheter wedged in the pulmonary arterial tree truly reflects the pulmonary venous pressure in patients who have pulmonary vascular changes.

That a decrease of left atrial pressure plays any part in the patients with pulmonary tuberculosis seems unlikely. In this group the pulmonary arterial pressures were normal or slightly elevated and there was no evidence of cardiac involvement. Euler and Liljestrand found that a drop in pulmonary arterial pressure in cats occurred with no change in left atrial pressure (1).

Because the range of pulmonary arterial pressures and arterial oxygen saturations in the tuberculosis group is not sufficiently great, no conclusions can be drawn as to what part anoxia may play in pulmonary arterial hypertension.

Although a majority of the patients in the pulmonary tuberculosis group had normal or only slightly elevated pulmonary arterial pressures, the tuberculous involvement was with few exceptions rather extensive. Pulmonary vascular changes have been reported in many of these individuals (21). Since it has been shown that oxygen breathing caused a statistically significant decrease in pressure in 14 patients with normal control pressures, the possibility exists that a similar decrease would occur in normal individuals.

It is recognized that significant differences exist between physiologic conditions at the altitude of Denver and those at sea level. The mean annual barometric pressure in Denver is 628 mm. Hg and the arterial oxygen tension is accordingly somewhat lower here. This somewhat lower oxygen tension may result in a greater degree of tonic activity of the chemoreceptors than exists at sea level. The effect of oxygen breathing on pulse rate, cardiac output and pulmonary vasculature might therefore be more evident in Denver. Possibly the effect of oxygen at this altitude is to abolish the slight relative anoxemia which normally exists here. If this is the case one might observe a less marked response at sea level or even no response at all (8).

The beneficial effect of oxygen therapy on patients with acute or chronic pulmonary arterial hypertension may depend not only upon the factors of increased arterial oxygen tension, decreased cardiac output, and decreased cardiac rate, but also upon a decreased pulmonary arterial pressure, a factor which would further reduce the work of the right ventricle.

SUMMARY

The effect of breathing 100% oxygen on the pulmonary arterial pressure in patients with pulmonary tuberculosis and mitral stenosis was studied 52 times in 38 patients.

1. Out of 27 studies in patients with pulmonary tuberculosis, 22 showed a decrease of the pulmonary arterial pressure with oxygen. This decrease was shown to be statistically significant.

2. Out of 25 studies in patients with mitral stenosis, 21 showed a decrease in pulmonary arterial pressure with oxygen. This decrease was shown to be statistically significant.

3. There was a definite correlation between the control mean pulmonary arterial pressure and the degree of its decrease.

4. The factors which may be involved in the observed decrease of pulmonary arterial pressure during oxygen breathing are discussed.

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PLASMA CHOLINESTERASE ACTIVITY IN LIVER DISEASE: ITS VALUE AS A DIAGNOSTIC TEST OF LIVER FUNCTION COMPARED WITH FLOCCULATION TESTS AND PLASMA PROTEIN DETERMINATIONS [1]

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The physiological significance of plasma cholinesterase is not clearly understood, but attention has recently been focussed on its relation to protein metabolism. It has been identified in high concentration in the α_2 globulin of Cohn's fraction IV-6-3 (1).

The evidence which has accumulated suggests that the liver is the probable source of plasma cholinesterase; and low values of plasma cholinesterase activity have been reported in patients with liver disease (2–11). We have been investigating acetylcholine and water metabolism in liver disease, and the present communication is an account of work undertaken to determine in which types of liver and biliary tract disease the plasma cholinesterase activity is deranged, and what diagnostic significance can be attached to these findings. Concurrent determinations of plasma proteins and flocculation tests were also made.

MATERIAL AND METHODS

The observations were carried out on 43 patients with liver and biliary tract disease. Of these, 15 patients were suffering from acute, four from sub-acute, 14 from chronic liver disease, and ten from extra-hepatic biliary obstruction. The plasma cholinesterase activity of 144 normal subjects was also studied; of these, 100 were healthy blood donors, and 44 were convalescent patients in hospital, who had no history or clinical evidence of liver disease; concurrent protein estimations were also made on the plasma of the latter patients.

Specimens of venous blood were transferred immediately after collection to heparinized tubes and centrifuged. After separation of the plasma, the red cells were washed twice with normal saline, and then laked with an amount of distilled water equal to the original volume of the plasma. The cholinesterase activity of each specimen of plasma and red cells was determined manometrically in duplicate at 38° C. by the method of Mendel, Mundell and Rudney (12), using the specific substrates, benzoylcholine chloride (0.006 M) for estimation of plasma cholinesterase activity, and acetyl-β-methylcholine chloride (0.03 M) for true cholinesterase. Cholinesterase activity is expressed as the amount of CO_2 in μL liberated in 60 minutes from 1 ml. of plasma, or laked red cell preparation.

Plasma protein estimations were made in duplicate, using the micro-Kjeldahl procedure. The globulin fraction was precipitated with 42 per cent sodium sulphite ($Na_2SO_3 \cdot 7H_2O$) by the method of King (13), and determinations were made of total protein and albumin. Concurrent flocculation tests were carried out on specimens of serum from all patients with liver and biliary tract disease, using the colloidal red (Maizels [14]), cephalin cholesterol flocculation (Hanger [15]), and thymol turbidity (Maclagan [16]) tests, according to the methods previously described by Maizels (14).

In assessing the value of plasma cholinesterase activity as a diagnostic test of liver and biliary tract disease a comparison has been made with the results obtained for plasma albumin and globulin and the serum flocculation tests. For the interpretation of the flocculation tests we have adopted as an index of abnormality the conventional standards used in this hospital (14), for the colloidal red and cephalin cholesterol tests, flocculation greater than one plus (+) at 24 hours, and for the thymol turbidity test a value greater than 4 units.

RESULTS

It will be convenient to begin by comparing the total results of the 33 patients with liver disease with those of the normal subjects in respect of plasma cholinesterase activity, plasma albumin and globulin estimations. Our aim here is to determine the limits of normality for these tests. The results have been plotted and give the overlapping histograms seen in Figure 1. The best discrimination will be given by the point on the scale where the two distributions (A) normal and (B) diseased intersect. A discrimination at this point

[1] The expenses of this work were defrayed partially by a grant from the Medical Research Council, from whom one of us (R. J. C.) is in receipt of a whole-time grant, and by financial assistance from the Ministry of Supply, from whom one of us (H. G.) receives a whole-time grant.

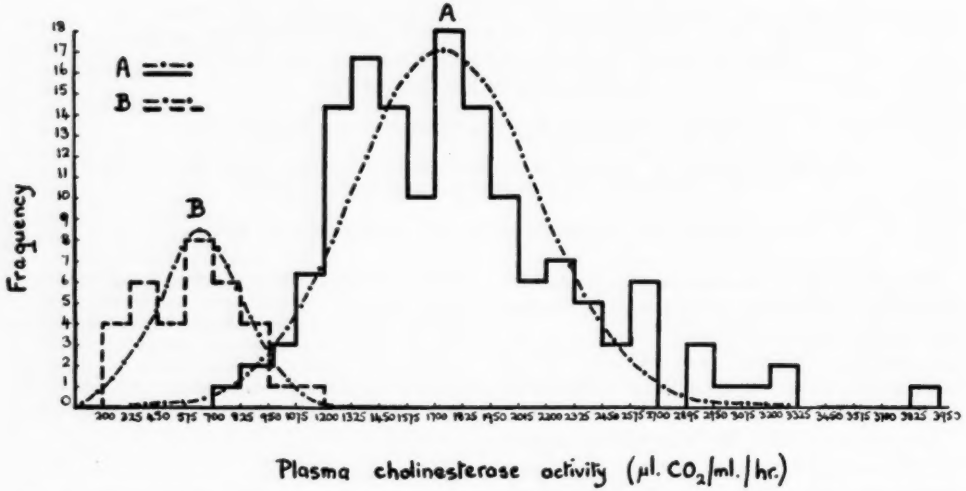

FIG. 1. FREQUENCY DISTRIBUTION OF VALUES FOR PLASMA CHOLINESTERASE ACTIVITY OF (A) 100 NORMAL SUBJECTS AND 44 CONVALESCENT PATIENTS IN HOSPITAL WITH NO EVIDENCE OF LIVER DISEASE AND (B) 33 PATIENTS WITH LIVER DISEASE

will yield a minimum number of misclassifications; that is, it will minimize the number of normal subjects (A) wrongly classified as abnormal (B), and of patients with liver disease (B) wrongly classified as normal (A). For purposes of calculating this discriminative point (Burt [17]),[2] it has been assumed in each case that the overlapping halves of the two samples are halves of two normal distributions. Since there was evidence that some of the distributions were slightly skewed, the measurements have been taken from the median rather than from the mean, and the standard deviations have been calculated from the adjacent or overlapping halves only.

The smoothed curve represents a hypothetical "normal distribution" having the same mean, standard deviation and number of cases as in the histogram. Since the asymmetry of the curves is not very great, the standard deviations calculated from the overlapping halves only have been used for the whole curves.

1a. Plasma cholinesterase activity

The values for normal subjects ranged from 818 to 3,265 with a mean value of 1,790. For the 33 patients with liver disease the mean value was 612, with a range of 238 to 1,086. These results have been plotted as a frequency distribution curve in Figure 1. The discriminative point is 936 and values less than this have been taken as an index of abnormality. The number of misclassifications thus produced will be 3.3 per cent of the total; 6.1 per cent liver disease patients being classified as normal, and 2.6 per cent of normal subjects as suffering from liver disease.

[2] The discriminating points given in the text were calculated by the formula

$$X = \frac{d\sigma_1^2 - \sigma_1\sigma_2\sqrt{d^2 + 2(\sigma_1^2 - \sigma_2^2)\log_e \frac{\sigma_1 N_2}{\sigma_2 N_1}}}{\sigma_1^2 - \sigma_2^2},$$

where σ_1 and σ_2 are the standard deviations of the two groups, N_1 and N_2, the numbers in the two groups, and d the difference between the medians.

This minimizes the total number of misclassifications. If it is argued that we should aim rather at equalizing the number (or the likelihood) of misclassifications in either group, then, as Burt has suggested, we should take

$$X = \frac{\sigma_1 N_1 \bar{x}_2 + \sigma_2 N_2 \bar{x}_1}{\sigma_1 N_1 + \sigma_2 N_2},$$

where $\bar{x}_1$ and $\bar{x}_2$ denote (as usual) the means for the two groups and $d = \bar{x}_2 - \bar{x}_1$. As calculation has shown, with our own data the two formulas lead to very much the same numerical values.

1b. Red cell cholinesterase activity

A quantitative analysis of the results fails to reveal any statistically significant differences between the red cell cholinesterase values of patients with liver disease and those of normal subjects.

1c. Plasma proteins

The mean value obtained for normal plasma albumin was 4.5 G. per cent (S.E. ± 0.06) and for plasma globulin, 2.7 G. per cent (S.E. ± 0.08). A calculation of the best discriminative point indicates that the lower limit of normality for plasma albumin may best be taken as 4.0 G. per cent and the corresponding upper limit for plasma globulin as 3.2 G. per cent. The discriminative point thus suggested for albumin values yields about 14.8 per cent misclassifications out of the total; 26.8 per cent of patients with liver disease, and 5.5 per cent of convalescent patients being wrongly classified. For globulin the borderline yields 16.7 per cent misclassifications out of the total; 29.1 per cent of liver disease patients and 7.1 per cent of convalescent patients being misclassified. It may be added that the usual statistical tests left no doubt that the discrimination was fully significant.

2. Patients with acute liver disease

This group of 15 patients including six (cases 1, 5, 8, 9, 10, 11) with homologous serum jaundice, was investigated in the icteric stage. All had typical signs and symptoms of acute virus hepatitis. Four patients were severely ill and are described as fulminating, three died within 48 hours of admission; 11 patients were moderately ill and were finally discharged from the hospital free from signs and symptoms. The diagnosis of five cases was confirmed by biopsy or autopsy. The results are summarized in Table I.

In the fulminating type the results of all tests were abnormal. There was, however, some divergence in agreement between the results for the moderately ill patients. Thus, although 82 per cent of these patients had abnormal values for plasma cholinesterase activity and positive flocculation tests, the cases in which the plasma cholinesterase activity was normal were different from the two with negative flocculation tests. The albumin and globulin levels were abnormal in only two cases. Considered as a whole, the flocculation tests and plasma cholinesterase activity are equally sensitive in indicating impairment of liver

TABLE I

Results of flocculation tests, plasma cholinesterase activity, plasma albumin and plasma globulin determinations on 15 patients with acute liver disease

Severity	Case no.	Flocculation tests				Plasma cholinesterase activity $\mu LCO_2/ml./hr.$	Plasma proteins	
		Coll. red	Ceph. chol.	Thymol turb. *units*	Collect.* interpretn.		Albumin G%	Globulin G%
Fulminating	1	++++	++++	12	positive	414	2.6	3.6
	2	+++	+++	12	positive	523	3.6	4.4
	3	+++	++++	10	positive	578	3.5	3.8
	4	++++	+++	9	positive	696	2.5	4.2
Nonfulmin.	5	++++	+++	18	positive	238	2.5	3.2
	6	++	++	5	positive	635	4.1	2.9
	7	++++	++++	7	positive	719	4.1	3.5
	8	++++	+++	8	positive	722	5.2	2.9
	9	+++	+++	12	positive	731	4.3	3.4
	10	+++	++++	8	positive	856	3.5	3.1
	11	0	+++	9	positive	928	4.4	2.1
	12	++++	++++	22	positive	968	4.3	2.9
	13	++	++	5	positive	1,086	4.3	2.7
	14	++	0	4	negative	834	4.5	3.1
	15	0	0	3	negative	792	4.8	2.0
Total no. with abnormal values					13	13	6	6

* The collective interpretation of the flocculation tests was assessed as positive where two or more tests were abnormal.

TABLE II

Results of flocculation tests, plasma cholinesterase activity, albumin and globulin determinations at the time of discharge from hospital in seven patients recovering (late) from acute liver disease, compared with the results obtained during acute illness (early)

Case no.	Phase	Flocculation tests				Plasma cholinesterase activity $\mu LCO_2/ml./hr.$	Plasma proteins		Interval between early and late tests *weeks*
		Coll. red	Ceph. chol.	Thymol turb. units	Collective* interpretn.		Albumin *G%*	Globulin *G%*	
1	Late	+++	+++	12	positive	2,708	3.9	3.2	6
	Early	++++	++++	12	positive	414	2.6	3.6	
5	Late	++	++	9	positive	1,383	4.0	3.2	6
	Early	++++	+++	18	positive	238	2.5	3.2	
6	Late	++	++	5	positive	1,381	3.8	2.8	2
	Early	++	++	5	positive	635	4.1	2.9	
7	Late	0	0	2	negative	1,490	4.2	3.2	9
	Early	++++	++++	7	positive	719	4.1	3.5	
8	Late	0	0	4	negative	917	—	—	4
	Early	++++	+++	8	positive	722	5.2	2.9	
12	Late	++++	+++	12	positive	1,508	4.5	3.0	1
	Early	++++	++++	22	positive	968	4.3	2.9	
13	Late	++	0	2	negative	1,532	4.3	3.3	5
	Early	++	++	5	positive	1,086	4.3	2.7	

* The collective interpretation of the flocculation tests was assessed as positive where two or more tests were abnormal.

function in patients with acute liver disease (87 per cent abnormal).

These tests were repeated on seven patients, at intervals until they were free from signs and symptoms of liver disease. A comparison is shown in Table II of the results obtained when the patients were acutely ill and when they had recovered sufficiently to be discharged from hospital. Whereas plasma cholinesterase values had returned to within normal limits in all but one of the patients, positive flocculation tests were still observed in four patients. Although the plasma globulin levels were normal in the acute phase as well as at the time of discharge, in some cases abnormal values were observed during the course of illness. In following up patients whose plasma cholinesterase and albumin levels were originally less than normal, it was not possible to conclude definitely whether plasma cholinesterase activity or albumin had first returned to normal.

TABLE III

Results of flocculation tests, plasma cholinesterase activity, albumin and globulin determinations on four patients with sub-acute hepatitis

Case no.	Flocculation tests				Plasma cholinesterase activity $\mu LCO_2/ml./hr.$	Plasma proteins	
	Coll. red	Ceph. chol.	Thymol turb. *units*	Collective* interpretn.		Albumin *G%*	Globulin *G%*
16	+++	+++	7	positive	280	1.8	3.6
17	++	+++	7	positive	469	3.5	3.5
18	+++	+++	9	positive	280	1.7	4.4
19	+++	+++	20	positive	447	3.9	4.0

* The collective interpretation of the flocculation tests was assessed as positive where two or more tests were abnormal.

3. Patients with sub-acute hepatitis

Four patients whose signs and symptoms had persisted for between two and four months are considered as a separate group; this is in general agreement with the classification of Kelsall, Stewart and Witts (18). All patients were jaundiced and had enlargement of the liver. Vascular spiders, and signs and symptoms involving the central nervous system, were noted in Cases 16 and 17, each of whom developed a terminal coma and died within a week of the observations. A diagnosis of sub-acute hepatic necrosis was confirmed at autopsy. One patient, Case 18, had one month previously recovered from hepatic coma, and Case 19 was admitted in relapse after treatment lasting two months in another hospital.

The severity of illness bore a striking resemblance to that of the patients with severe acute liver disease, and the results of the tests, shown in Table III, are very similar to those shown in Table I. Plasma cholinesterase levels were all extremely low; strongly positive flocculation tests, low plasma albumin and high globulin levels were also observed. Serial estimations of plasma cholinesterase activity in the two patients who survived (Cases 18 and 19), reflected the gradual process of recovery in advance of the changes shown by the other tests.

4. Patients with chronic liver disease

The 14 patients included in this group consisted of five cases of chronic hepatitis, seven of portal cirrhosis, one with primary biliary cirrhosis, and one with congestive cirrhosis of the liver. The patients with chronic hepatitis had a history of an acute attack with jaundice, and were observed at a period of about one year after the acute attack; the clinical evidence of liver disease was as marked as in the patients with portal cirrhosis. Jaundice was present in about a quarter of the patients. Portal hypertension was a feature of the group with portal cirrhosis, but was also frequently observed in patients with chronic hepatitis. There was clinical evidence of water retention in approximately two-thirds of the patients in each group. The clinical diagnosis was confirmed by biopsy or autopsy in ten patients.

Low plasma cholinesterase values were observed in all the patients; in contrast, the flocculation tests were negative in more than half the cases of portal cirrhosis (Table IV). In general, low plasma albumin levels were observed, but the plasma globulin was raised in only about half of the patients with portal cirrhosis. With regard to the flocculation tests and plasma globulin values, there appears to be a clear distinction between patients with chronic hepatitis and those with portal cirrhosis, but the reasons for this are obscure. As a diagnostic measure, however, it is obvious that the estimation of plasma cholinesterase activity provides a more sensitive index of impairment of liver function than is obtained with other tests.

5. Patients with extra-hepatic biliary obstruction

Ten patients with extra-hepatic biliary obstruction were studied, and in all but one (Case 43), the diagnosis was confirmed at operation or autopsy. One patient (Case 34) was observed during his fifth attack of biliary colic and although previous attacks had usually been associated with

TABLE IV

Results of flocculation tests, plasma cholinesterase activity, plasma albumin and globulin determinations on 14 patients with chronic liver disease

Diagnosis	Case no.	Flocculation tests				Plasma cholinesterase activity $\mu LCO_2/ml./hr.$	Plasma proteins	
		Coll. red	Ceph. chol.	Thymol turb. *units*	Collective* interpretn.		Albumin *G%*	Globulin *G%*
Chronic hepatitis	20	++++	+++	13	positive	290	1.9	3.8
	21	+++	+	8	positive	354	2.7	3.5
	22	+++	+	12	positive	418	2.4	4.3
	23	+++	+++	11	positive	425	2.9	4.4
	24	+++	++++	10	positive	466	4.0	4.2
Portal cirrhosis	25	++++	++	17	positive	671	4.0	3.9
	26	++++	+++	12	positive	676	3.5	4.2
	27	++++	+++	15	positive	790	2.8	4.2
	28	0	0	3	negative	559	3.1	2.3
	29	0	0	3	negative	578	3.9	3.1
	30	0	+	3	negative	635	3.2	3.0
	31	++	0	3	negative	891	3.0	4.5
Primary biliary cirrhosis	32	++	++	3	positive	735	3.4	3.6
Congest. cirrhosis	33	+	0	3	negative	638	3.7	2.9

* The collective interpretation of the flocculation tests was assessed as positive where two or more tests were abnormal.

TABLE V

Results of flocculation tests, plasma cholinesterase activity, plasma albumin and globulin determinations on ten patients with extra-hepatic biliary obstruction

Diagnosis	Case no.	Duration of jaundice *weeks*	Flocculation tests				Plasma cholinesterase activity *μLCO₂/ml./hr.*	Plasma proteins	
			Coll. red	Ceph. chol.	Thymol turb. *units*	Collective* interpretn.		Albumin *G%*	Globulin *G%*
Choledocholithiasis S-A cholangitis	34	0	0	0	2	negative	1,700	4.5	2.9
Choledocholithiasis-acute cholangio-hepatitis	35	1	+	0	2	negative	873	4.2	1.9
Recurrent biliary obstruction. Polyp in duodenum near ampulla of Vater	36	1	0	0	1	negative	1,956	4.5	2.6
Carcinoma of head of pancreas	37	1	0	0	1	negative	2,293	3.9	3.1
Choledocholithiasis adenocarcinoma of gallbladder	38	5	0	0	1	negative	1,209	4.4	2.7
Carcinoma of ampulla of Vater	39	8	0	0	2	negative	1,461	3.2	3.7
Carcinoma of ampulla of Vater	40	9	0	0	5	negative	828	3.6	3.4
Carcinoma of common bile duct; extensive secondary hepatic metastases	41	22	0	0	1	negative	657	3.3	3.9
Choledocholithiasis	42	12	0	0	2	negative	1,675	3.8	3.7
Recurrent cholangio-hepatitis	43	32	0	0	4	negative	1,086	4.3	4.4

* The collective interpretation of the flocculation tests was assessed as positive where two or more tests were abnormal.

jaundice, on the present occasion this was not observed until the day following the tests; at operation a stone was demonstrated in the cystic duct, occluding the common bile duct.

The results of the flocculation tests, plasma cholinesterase, albumin and globulin estimations are shown in Table V. The cases are arranged consecutively in order of the duration of jaundice which is indicated in column *2*; for convenience of subsequent discussion the table is subdivided into early and late cases. The flocculation tests in all cases were consistently negative and in this respect are in marked contrast to all the other tests, in distinguishing jaundice due to extra-hepatic biliary obstruction from that due to liver disease. The plasma cholinesterase values were normal in seven patients. Of the three patients with low values one (Case 36) had an acute attack of cholangiohepatitis; the other two (Cases 40 and 41) had a history of prolonged obstruction, and had subsequent evidence of malignant disease. Plasma albumin levels were normal in all but one of the early group and abnormal in all but one of the late group; essentially similar findings were obtained for plasma globulin levels.

DISCUSSION

It has been suggested that the low plasma cholinesterase activity observed in patients with liver disease may be due to an inhibitor substance (3), but there is no satisfactory evidence to support this hypothesis (4). In a series of experiments involving incubation of normal plasma with that from patients with liver disease, we have been unable to demonstrate an inhibitory effect; using techniques previously described (19) we have not observed any difference from normal in the plasma cholinesterase of patients with liver disease with respect to optimum substrate concentration or the *in vitro* inhibition produced by the anti-cholinesterase compounds di-isopropylfluorophosphonate and neostigmine.

We have explored the variations in plasma cholinesterase activity of patients with acute, subacute and chronic liver disease, and have attempted to define more precisely the value of this test in comparison with the flocculation tests and plasma albumin and globulin levels. The collective information obtained from all these patients has confirmed the reliability of a low value for plasma cholinesterase activity as an index of impaired liver function. In severe acute liver disease this test does not contribute more information than is obtained by the other tests, but in patients who are less severely affected, the test is as sensitive as the flocculation tests, and much more reliable than estimations of plasma albumin and globulin. Grob and his colleagues (8) have drawn attention

to the apparent lack of precision of this test in acute as compared with chronic liver disease. Vorhaus, Scudamore, and Kark (20) on the other hand have commented on the reliability of plasma cholinesterase estimations as an index of biochemical relapse in following up patients with acute hepatitis, in contrast to other liver function tests. We have also shown that, while normal values for plasma cholinesterase activity may be obtained in conjunction with positive flocculation tests, this apparent anomaly is indeed an indication of the immediate prognostic value of the test, since it reflects in advance of the flocculation tests the phase of recovery. A question of considerable interest is whether at the beginning of an acute attack of liver disease, depression of plasma cholinesterase activity occurs before, concurrently with, or after the development of positive flocculation tests.

Our observations on patients with sub-acute liver disease are restricted to four cases in whom all tests were positive; plasma cholinesterase estimations merely confirm the severity of this type of liver disease.

We have shown, as other workers have observed (21–24), that the flocculation tests are often negative in patients with chronic liver disease. We have found that this is evident particularly in those cases with portal cirrhosis, three of which had negative flocculation tests during intermittent observations throughout a period of 12 months; in contrast, low values for plasma cholinesterase activity were observed in all patients. Kunkel and Ward (9) have already drawn attention to the close relation between the low plasma albumin and low plasma cholinesterase values in chronic liver disease, and we have confirmed this by serial estimations carried out on several of our patients over periods of 12 months or more. We have failed to observe any such marked relation, however, in acute liver disease, and we consider that the conclusions of Levine and Hoyt (25) do not merit the assumption that if the serum albumin level is low, the serum cholinesterase level must also be low. Our results taken in conjunction with those of Kunkel and Ward (9) and Alcalde (11) justify the conclusion that chronic liver disease is almost invariably associated with low plasma cholinesterase activity.

The original observation by McArdle (4) that a high proportion of patients with extra-hepatic biliary obstruction have initial normal serum cholinesterase values, has been confirmed by most workers; in this respect our results are also in agreement, for, of ten patients, seven had normal plasma cholinesterase levels. There are several factors which limit the value of this test in distinguishing between jaundice due to hepatic and extra-hepatic biliary causes. One is the duration of jaundice, which, if prolonged beyond about two months, may result in low values; this was commented on by Grob and his colleagues (8) who observed low values in four patients with prolonged obstructive jaundice. Malignant disease may also produce a depression of plasma cholinesterase activity; Alcalde (11) has shown that whereas 26 patients with benign obstructive jaundice had mainly normal values, all of the 24 patients with malignant obstruction had low values. How far these low values in malignant obstruction are due directly to impairment of liver function is not clear; only two of our patients in the obstructive group had low values, yet five cases had evidence of malignant disease at operation or subsequent autopsy. Intercurrent infection of the biliary tract may also produce a transient lowering of plasma cholinesterase activity; this we have observed on several occasions during serial estimations in patients with cholangiohepatitis (Himsworth [26]).

There appears to be little relation between plasma globulin levels and the flocculation tests in extra-hepatic biliary obstruction, for in contrast to the uniformly negative flocculation tests, we have observed consistently raised plasma globulin levels when the obstruction is prolonged.

As an aid in the differential diagnosis of jaundice, estimations of plasma cholinesterase activity are less precise than the flocculation tests in confirming the extra-hepatic biliary group, but are particularly valuable in assessing concurrent impairment of liver function. When used in conjunction with the flocculation tests, they afford an immediate prognostic index of recovery in acute and subacute liver disease. The estimation of plasma cholinesterase activity is probably the most direct and sensitive method of assessing the ability of the liver to synthesize protein, and the test re-

flects the severity of the clinical condition of patients with liver disease. The chief criticism of this test lies in the specialized technique required, but in laboratories not equipped for this purpose, the simplified method described by Michel (*27*), which depends on measuring changes in pH, offers, as Vorhaus, Scudamore and Kark (10) have shown, an alternative and reliable technique.

SUMMARY AND CONCLUSIONS

1. The plasma cholinesterase activity of 100 normal subjects, 44 convalescent hospital patients with no history or clinical evidence of liver disease, and of 33 patients with liver disease has been studied. It has been shown that, compared with normal subjects and the convalescent patients, there is a significant difference in the plasma cholinesterase activity of patients with liver disease.

Values for plasma cholinesterase activity lower than 936 (μLCO_2/ml./hr.) are regarded as abnormal.

2. The results of plasma cholinesterase determinations have been compared with those of the flocculation tests and of plasma albumin and globulin estimations in 15 patients with acute, 4 patients with sub-acute, and 14 patients with chronic liver disease and in 10 patients with extra-hepatic biliary obstruction. It has been shown that in patients with severe acute and sub-acute liver disease, low plasma cholinesterase values provide confirmatory evidence of considerable impairment of liver function. In patients recovering from acute and sub-acute liver disease, estimations of plasma cholinesterase activity afford an immediate useful prognostic indication of recovery of liver function, since the plasma cholinesterase level returns to within normal limits in advance of the flocculation tests.

It has been observed that in patients with chronic liver disease, where the flocculation tests are sometimes normal, plasma cholinesterase and albumin levels are usually lower than normal. This evidence, however, does not appear to discriminate between cirrhosis and malignant disease of the liver.

3. Normal values for plasma cholinesterase activity are usually observed in patients with extra-hepatic biliary obstruction of short duration; low values occur, however, during attacks of cholangio-hepatitis and in patients with malignant disease and prolonged obstruction. In the differential diagnosis of jaundice the results of this test are less precise than those of the flocculation tests in defining an extra-hepatic cause for the jaundice, but when taken in conjunction with the flocculation tests provide reliable information regarding impairment of liver function.

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CEREBRAL CIRCULATION AND METABOLISM IN SICKLE CELL AND OTHER CHRONIC ANEMIAS, WITH OBSERVATIONS ON THE EFFECTS OF OXYGEN INHALATION [1]

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Sickle cell anemia may be associated with a variety of serious cerebral manifestations, such as hemiplegia, aphasia, convulsions, stupor or coma (1, 2). These symptoms are usually attributed to thromboses of the cerebral vessels produced by sickled erythrocytes, vascular stasis and degenerative changes in the small and medium-size arteries of the central nervous system (3, 4). In chronic anemias from various causes, such mental manifestations as drowsiness, irritability, and fainting are often seen. Psychoses have been observed in pernicious anemia and in chronic posthemorrhagic anemia (5). A diminution in cerebral oxygen consumption in patients with pernicious anemia has been reported and may be the basis for the alterations in the mental status seen in these patients (6). It seemed worth-while for these reasons to study the cerebral circulation and metabolism in such patients, and this report presents the findings in 10 patients with sickle cell disease and eight patients with other types of chronic anemia. In order to obtain insight into the pathogenesis of the abnormalities observed, the effects of inhalation of 85–100% oxygen on cerebral functions were determined and the data thus obtained are also presented in this report.

CASE MATERIAL AND METHODS

The ages of the 10 patients with sickle cell anemia in this study varied from 13 to 34 years with an average of 24 years. All of the patients were Negroes; two were males and eight were females. Two of the patients (E. L., A. F.) had the onset of acute sickle cell crises several days prior to the cerebral studies. The remaining eight patients were in remission at the time of the studies. Evidence of sickle cell anemia had been present in all 10 patients since childhood and included such symptoms as arthralgia, fever, abdominal pain, easy fatigability and, occasionally, dyspnea. All of them showed pallor of the mucous membranes, scars of old pretibial ulcers and dilatation of the retinal veins. Four patients had X-ray evidence of cardiac enlargement, but none had symptoms of congestive failure. Neurologic manifestations or evidence of mental retardation had not been observed.

Sherman's method for the determination of the percentage of sickled cells was employed (7). The number of abnormal cells in the internal jugular venous blood at the time of the cerebral studies ranged from 7% to 43%, with a mean of 21%. The number of true sickled cells was considerably less and averaged 3.6%.

The group of eight patients with other types of chronic anemia comprised one patient (D. C.) with aplastic anemia, three with iron-deficiency anemia (R. C., E. T., O. J.), one with pernicious anemia (L. H.), and three with anemia caused by chronic blood loss from either hemorrhoids or menorrhagia (W. S., L. B., K. C.). The duration of anemia in these individuals ranged from six months to 11 years. They showed no manifestations of mental deterioration, neurologic involvement or congestive failure.

Ten patients convalescing from a variety of illnesses, such as gonorrheal arthritis, dermatitis and peptic ulcer, and from operations such as herniorrhaphy, were used as control subjects. These patients had only slight or no anemia and no evidence of intracranial or pulmonary complications.

The cerebral blood flow (CBF) was measured by the nitrous oxide method with minor modifications previously described (8, 9). The cerebral metabolic rate ($CMRO_2$) was calculated from the CBF and the cerebral arteriovenous oxygen difference. The cerebral vascular resistance (CVR) was calculated from the CBF and the mean arterial pressure measured directly from the femoral artery with a damped mercury manometer. Control observations of the cerebral circulation were performed with the patient breathing 21% oxygen, 15% nitrous oxide, and 64% nitrogen, following which the subject was given 100% oxygen by face mask for a period of 20 minutes. At the end of this time the gas was changed to 85% oxygen and 15% nitrous oxide, and a second blood flow determination carried out.

Samples of arterial and venous blood drawn just before and at the end of each blood flow procedure were pooled, and their O_2 and CO_2 content was measured by the combined procedure described by Peters and Van Slyke (10). The nitrous oxide content of blood sam-

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ples was determined by the manometric method of Orcutt and Waters (11). Modifications of these gasometric technics, suggested by Kety and Schmidt (8), were employed. Oxygen capacity of blood was obtained by equilibrating the sample with air in a small flask, analyzing for oxygen manometrically, and making the usual correction for dissolved oxygen. Hemoglobin concentration was calculated from the oxygen capacity. Arterial pH was measured with the glass electrode of a Cambridge Model R pH meter and corrected to body temperature (12). Arterial pCO_2 was calculated from the pH, CO_2 content and hematocrit by means of the nomogram of Singer and Hastings (13). Venous pO_2 was calculated from the pH and the per cent oxyhemoglobin saturation, using the dissociation curves of Dill (14).

RESULTS

The mean cerebral blood flow in the patients with sickle cell anemia was found to be 72 cc./100 gm. brain/min., a value considerably higher ($p < .001$) than the CBF of 49 cc. observed in the control patients (Table I, Figure 1). The patients with sickle cell anemia showed a wide range of CBF values from 50 to 91 cc. The eight patients with other types of chronic anemia had a mean CBF of 64 cc., a value significantly higher than the controls ($p < .02$). There was some correlation between the hemoglobin level and the CBF in the sickle cell patients ($r = .66$). The three patients (H. W., J. H., L. G.) with more than 10 gm. of hemoglobin per 100 cc. had a mean CBF value of 58 cc., while the seven patients with hemoglobin values of 5 to 7.7 gm. had a mean CBF of 78 cc.

Mean arterial pressure in both groups of anemic patients differed little from that found in the control subjects. The mean CVR of 1.3 mm. Hg/cc./100 gm./min. in sickle cell anemia was significantly reduced below the control value. The mean CVR in the chronic anemias of other etiology was 1.5 mm. Hg, a value slightly higher than that found in the sickle cell patients.

The mean $CMRO_2$ of the patients with sickle cell anemia was moderately reduced below that found in the control subjects and averaged 2.4 cc./100 gm. brain/min. ($.05 > p > .02$). The two patients with the highest hemoglobin values (10.6 and 11 gm.) had normal values for cerebral oxygen consumption, while the patient (J. H.) with 10 gm. of hemoglobin had the low $CMRO_2$ value of 2.1 cc. The mean arteriovenous oxygen difference in the sickle cell group was 3.5 vol. %, in contrast to the control value of 5.9 vol. %. A close correlation was observed between the arteriovenous oxygen difference and the hemoglobin values in the individual patients. The mean $CMRO_2$ in the eight patients with other types of chronic anemia was 2.3 cc./100 gm./min., a value comparable to that found in the sickle cell patients, but less than that of the controls ($.1 > p > .05$). Two of the three patients in this group with normal values for cerebral oxygen consumption (3 cc. or more) were young women (K. C., L. B.) with blood loss caused by menorrhagia. The third patient (E. T.) had anemia associated with nutritional deficiency. The mean value for jugular venous oxygen tension in the patients with sickle cell anemia was 28 mm. Hg, or 5 mm. Hg less than that found in the control subjects. The mean arterial and venous CO_2 tensions were both slightly lower in these patients than in the controls. The mean jugular venous pO_2 in the patients with other types of chronic anemia was found to be 28 mm. Hg.

During inhalation of 85–100% oxygen all of the patients with anemia showed decreases in

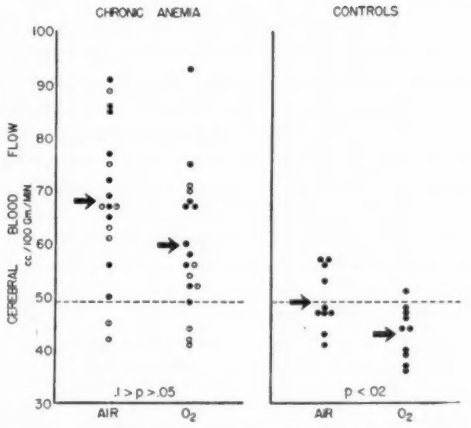

FIG. 1. VALUES FOR CEREBRAL BLOOD FLOW IN INDIVIDUAL PATIENTS WITH CHRONIC ANEMIA AND IN CONTROL SUBJECTS BEFORE AND DURING ADMINISTRATION OF 85–100% OXYGEN

The solid dots in the anemia group represent patients with sickle cell anemia, and the open circles, patients with other types of anemia. The arrows indicate mean values in each group. The dotted line represents the average CBF in the control subjects breathing air.

TABLE I

Physiologic data in patients with anemia

Pt.	Age	CBF (*cc./100 gm./min.*)		$CMRO_2$ (*cc./100 gm./min.*)		CVR (*mm. Hg/cc./100 gm./min.*)		Mean Art. Pressure (*mm. Hg*)		A-V Oxygen (*vol. %*)		Hematocrit	Hemoglobin (*gm./100 cc.*)	Art. pH		Art. pCO_2 (*mm. Hg*)		Venous pCO_2 (*mm. Hg*)		Venous pO_2 (*mm. Hg*)	
		Air	O_2	Air	O_2	Air	O_2	Air	O_2	Air	O_2			Air	O_2	Air	O_2	Air	O_2	Air	O_2
Sickle cell anemia																					
H. W.	28	67	58	3.3	3.1	1.4	1.7	91	96	4.9	5.3	31.7	11.0	7.39	7.38	39	38	50	52	33	40
L. G.	14	56	52	3.2	3.7	1.7	1.6	95	83	5.7	7.2	31.0	10.6	7.42	7.36	39	43	51	58	29	31
J. H.	30	50	49	2.1	2.8	1.9	1.8	92	86	4.3	5.7	30.4	10.0	7.36	7.40	36	34	46	43	31	33
H. L.	19	69	75	2.5	3.6	1.2	1.2	85	91	3.7	4.8	24.0	7.7	7.35	7.36	—	38	—	46	25	39
F. B.	34	85	60	2.1	2.1	1.4	1.7	117	102	2.5	3.5	20.8	6.5	7.40	7.43	34	30	35	37	24	35
E .L.	22	72	67	2.5	2.7	1.2	1.4	87	92	3.4	4.0	19.0	5.4	7.40	7.38	33	34	40	39	21	26
R. M.	22	86	93	1.9	3.6	1.0	.9	82	84	2.3	3.9	22.0	7.0	7.37	7.42	41	36	45	45	30	33
M. A.	23	65	67	1.6	2.3	1.5	1.4	98	96	2.5	3.4	17.7	6.1	7.35	7.42	39	33	40	47	29	42
D. C.	22	77	68	2.6	3.1	1.1	1.3	82	86	3.3	4.6	24.3	7.5	7.43	7.42	39	37	44	47	30	33
A. F.	22	91	56	2.3	1.9	1.0	1.6	92	88	2.5	3.4	20.0	6.1	*	*	36	35	41	43	26	34
Mean	24	72†	65†	2.4‡	2.9	1.3†	1.5†	92	90	3.5	4.6	24.1	7.8	7.39	7.40	37	36	44	46	28	35
Std. Dev.		13	12	0.5	0.6	0.3	0.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Std. Err.		4.0	3.9	0.2	0.2	0.1	0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chronic anemia of other etiology§																					
D. C.	57	42	42	1.8	2.6	2.1	2.1	88	89	4.4	6.1	22.8	7.0	7.37	7.40	36	33	45	47	26	24
R. C.	21	67	52	2.2	2.0	1.3	1.6	86	83	3.2	4.0	21.2	5.6	7.31	7.33	39	38	47	50	28	29
L. H.	66	45	41	1.2	1.6	2.7	2.6	120	106	2.7	3.9	22.0	7.3	7.36	7.38	41	41	50	53	35	34
W. S.	53	63	44	1.7	1.6	1.4	1.9	86	82	2.7	3.6	16.2	4.3	7.35	7.39	43	39	46	47	22	26
K. C.	17	61	70	3.0	4.1	1.1	1.0	64	71	4.9	5.9	30.4	8.0	*	*	43	45	46	55	25	29
L. B.	26	75	54	3.4	3.2	1.1	1.5	84	82	4.5	5.9	29.9	8.0	*	*	41	38	49	50	25	25
E. T.	53	89	71	3.4	3.5	0.9	1.0	79	70	3.8	4.9	22.4	7.5	*	*	45	44	52	55	28	30
O. J.	65	67	56	1.8	2.2	1.3	1.8	85	99	2.7	3.9	14.1	4.4	*	*	41	43	49	—	24	25
Mean	45	64	54	2.3	2.6	1.5	1.7	87	85	3.6	4.8	22.4	6.5	7.35	7.38	41	40	48	51	27	28
Mean 10 Control Pts.	31	49	43‖	2.9	3.1	1.9	2.1	93	91	5.9	7.1	40.2	13.1	7.37¶	—	42¶	—	50¶	—	33¶	—
Std. Dev.		5.6	4.7	0.5	0.5	0.4	0.3	—	—	—	—	—	—	.03	—	3.2	—	2.7	—	3.8	—
Std. Err.		1.8	1.5	0.1	0.2	0.1	0.1	—	—	—	—	—	—	.01	—	1.0	—	0.8	—	1.1	—

* pH not obtained. The pO_2 and pCO_2 calculations in these patients were made with the means of the pH values of all of the other anemia patients.

† Statistically significant difference from mean value in *control* patients breathing gas of same composition.

‡ Significance of difference from control mean: $.05 > p > .02$.

§ Because "chronic anemia of other etiology" constitutes a heterogeneous group, the mean values are not compared statistically with mean values of the sickle cell group.

‖ Statistically significant difference ($p < .02$) from mean value in *same* patients breathing room air.

¶ Data from 11 convalescent patients; 3 of these are represented in the CBF data.

CBF and increases in CVR comparable to those found in the controls. Mean arterial pressure tended to diminish slightly in all groups. Despite the reduction in CBF, the patients with sickle cell disease had a moderate increase in $CMRO_2$ from a mean of 2.4 to 2.9 cc. with oxygen inhalation ($.1 > p > .05$). This change was associated with an increase in arteriovenous oxygen difference from 3.5 to 4.6 vol. %.

The eight patients with other types of chronic anemia also had a small mean increase in cerebral metabolism during oxygen administration. This change in $CMRO_2$ from 2.3 to 2.6 cc./100 gm./min. was statistically insignificant. The control subjects also had little change in $CMRO_2$ with oxygen. The mean venous pO_2 rose from 28 to 35 mm. Hg in the patients with sickle cell anemia during oxygen inhalation, but did not change in the individuals with other types of anemia.

DISCUSSION

It is probable that the elevation of the cerebral blood flow observed in patients with chronic anemia resulted from a combination of factors. Since the arterial pressure in these patients differed only slightly from that in the controls, it is evident that vascular resistance was diminished. This could have been caused in part by the decrease in blood viscosity which resulted from the anemia (15), or by an increase in the caliber of the cerebral vessels.

Reduction in blood oxygen tension in the brain could have provided the stimulus for vasodilatation. The arterial oxygen tension has been found by Hickam (16) to be somewhat reduced in patients with severe anemia. It is evident that, unless a reduced arterial oxygen content is matched by an equivalent increase in blood flow, further abnormalities must follow: either the mean capillary and venous O_2 tensions will undergo further decreases relative to the normal levels, or cerebral oxygen consumption will fall, or both. Actually, both conditions were found. It is not certain whether reduced capillary or venous pO_2 per se represents a vasodilator stimulus, but it has been shown that a decrease in arterial oxygen saturation, produced by the inhalation of low-oxygen mixtures, results in cerebral vasodilatation (17). Changes in pO_2 could not have been the only factor in producing the lowered vascular resistance in the patients with sickle cell anemia, since the cerebral blood flow failed to return to normal with the rise in venous pO_2 to normal during oxygen inhalation.

The tendency for cerebral oxygen consumption to be reduced in the patients with chronic anemia, regardless of the etiology, raises the possibility that anemia itself can bring this about. The reduced venous pO_2 found in the anemic patients is evidence that the cerebral tissue oxygen tension was also below normal. Although rapid reduction in arterial and jugular venous oxygen tension by the inhalation of 10% oxygen produces little or no change in cerebral metabolism (17), our observations suggest that prolonged reduction in tissue oxygen tension, produced by chronic anemia, may have an adverse effect on cerebral function. The increase in $CMRO_2$ observed in several of the patients during oxygen inhalation lends some support to this view. The nerve cells of the brain in these patients were evidently capable of metabolizing at a higher level when presented with oxygen at a higher tension. Increase in $CMRO_2$ during oxygen administration was not observed in the control subjects, or in our patients with cerebral vascular disease associated with hypertension and arteriosclerosis (18), or in patients with emphysema (19).

The extremely low $CMRO_2$ values in some of the patients with sickle cell and other chronic anemias was rather surprising, in view of the normal mental state of the individuals. All of them were oriented, reasonably alert, and showed no neurologic or mental abnormalities. These observations are in contrast to those made in patients with pernicious anemia, in whom there appeared to be some correlation between abnormal mental status and cerebral oxygen consumption (6). It was believed, however, that in these patients the reduction in $CMRO_2$ was not related so much to the degree of anemia as to the deficiency state causing pernicious anemia.

The fact that the CBF was high in two patients at the time of severe sickle cell crises is difficult to reconcile with the prevalent belief that vascular stasis occurs in a large proportion of cerebral vessels during this period and is the cause of neurologic manifestations. Although vascular thrombosis associated with circulatory stasis undoubtedly occurs in a number of these patients, it is also possible that many of the cerebral manifestations of sickle cell disease are caused by chronic hypoxia of the brain.

It is apparent from the present studies, as well as those of Scheinberg (6), that the central nervous system suffers more profoundly in anemia than is generally appreciated.

SUMMARY

1. The cerebral blood flow (CBF), cerebral oxygen consumption ($CMRO_2$), and cerebral vascular resistance (CVR) were determined by the nitrous oxide technic, before and during the administration of 85–100% oxygen, in 10 patients with sickle cell anemia and eight patients with chronic anemia of other types.

2. The mean CBF in the patients with anemia as a group was significantly elevated, while the mean $CMRO_2$ was moderately reduced below that of control subjects. There were only small differences between the mean values for cerebral blood flow and oxygen consumption in the two groups of anemic patients.

3. The administration of 85–100% oxygen in the patients with anemia was generally followed by a decrease in the CBF and an increase in CVR comparable to those found in the control subjects. The $CMRO_2$ in many of the patients with anemia showed a rise during oxygen inhalation.

4. It is believed that the increase in cerebral blood flow in chronic anemia is caused by the combined effect of reduction in blood viscosity and vasodilatation from reduction in arterial, mean capillary and venous oxygen tensions. It is further suggested that the reduction in cerebral metabolism and possibly to some extent the mental symptoms observed in chronic anemia may be due to chronic tissue hypoxia. The increase in cerebral metabolism with oxygen inhalation in a number of the patients with anemia suggests that the use of oxygen may be a rational emergency therapeutic measure when mental symptoms are present.

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EFFECT OF ACTH ON WATER DISTRIBUTION IN MAN AS MEASURED BY ANTIPYRINE, T 1824 AND BROMIDE [1]

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The retention of salt and water by patients receiving ACTH and cortisone is well known (1). If sufficiently marked, rapid weight gain, edema and pulmonary congestion may result. It was considered of interest, therefore, to study the changes in plasma volume, extracellular fluid volume, total body water, and total body chloride occurring in patients receiving adrenocorticotropic hormone. A transient increase in the inulin space in patients treated with ACTH and cortisone and maintained on rigid salt restriction has been reported recently by Levitt and Bader (2).

METHODS

Five patients were studied; three had rheumatoid arthritis, one rheumatoid spondylitis, and one rheumatic fever with polyarthritis. Three patients were males and two females; their ages ranged between 28 and 53. In order not to limit the magnitude of the expected physiological changes, salt intake was not restricted. The amount of salt taken by shaker along with the regular hospital diet was, however, weighed daily, and proved to be relatively constant for each patient, varying between 1 and 3 grams per day.

Following a preliminary control study, the patients were treated with ACTH in dosages of 100 to 120 mg. per day [3] for about four weeks. Three patients, E. S., M. U., and G. R., received a supplement of 3 grams of KCl per day. Volume studies, determination of serum Na^+, Cl^- and HCO_3^- levels, complete blood and eosinophil counts, and determination of E.S.R. and hematocrit were done at approximately weekly intervals during treatment and also at intervals varying from six to 14 days following the end of ACTH administration.

T 1824 space, antipyrine space and bromide space were measured simultaneously; total body chloride was estimated.

T 1824 space: Plasma volume was measured by the T 1824 dye method (3). Blood samples were taken 10 and 20 minutes following dye administration.

Antipyrine space: This was determined by the method of Soberman and his associates (4a). Approximately 1.5 grams of antipyrine [4] were infused simultaneously with sodium bromide from a calibrated burette. The exact amounts of antipyrine as well as of bromide infused were established by direct analysis of the infused solutions. In non-edematous patients, samples were taken at two, three, four and five hours. In the two patients who developed significant peripheral edema, samples were taken at three, five, seven, and eight hours. It was demonstrated that the presence of T 1824 dye in the plasma did not increase the ultraviolet absorption of the plasma filtrates at 350 mμ after nitrosation, and it was concluded from this that T 1824 does not interfere with the determination of antipyrine.

Bromide space: This was measured by the method of Brodie and Friedman (5). From 1.5 to 2 grams of bromide were infused as sodium bromide in 2% solution with the antipyrine. Correction for Donnan equilibrium and plasma water concentration was made by dividing the serum concentration by the factor 0.88. Fusion of the plasma samples eliminated possible interference from T 1824 and antipyrine in the analysis of bromide. When known amounts of bromide were added to plasma, 98 to 102% were recovered.

Brodie, Brand, and Leshin (6) have shown that bromide arrives at diffusion equilibrium in humans within 2.5 hours and Berger and his coworkers (7) have demonstrated that bromide equilibrates between plasma and pleural fluid in dogs in two to four hours. The latter authors and Dunning, Steele, and Berger (8) analyzed five hour blood samples in non-edematous patients. Eliel, Pearson, and Rawson (9) averaged the four and five hour samples. Bromide excretion has been neglected during the five hour period in the investigations cited because it comprises approximately 1% of the administered bromide (9). In order to establish the time of diffusion equilibrium of bromide, measurements of the bromide space were made in eight subjects not previously included; five were given no hormone, two received ACTH

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[2] Now at the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

[3] This was pork ACTH, kindly supplied by Armour and Co. Dosage is expressed as milligrams equivalent to Armour Standard LA-1-A.

[4] This substance was kindly provided in sterile ampules by Eli Lilly and Co.

TABLE I

Bromide space at varying intervals after infusion in untreated and ACTH treated patients to whom bromide had not been previously administered

Patient	Age	Sex	Wt.	Disease	Treatment	Daily dosage	Days	Bromide space (*liters*)			
								4 hr.	5 hr.	7 hr.	24 hr.
	yrs.		*Kg.*			*mg.*					
K. S.	29	F	34.4	Osteoarthritis	None	X	X	—	10.6	10.6	10.7
W. C.	42	F	50.3	Rheumatoid arthritis	None	X	X	—	13.7	13.7	14.0
T. T.	14	M	28.4	Rheumatoid spondylitis	None	X	X	—	10.8	10.9	10.1
C. E.	22	M	73.6	Anal fissure	None	X	X	—	19.0	18.8	18.6
A. S.	58	M	64.6	Inguinal hernia	None	X	X	—	13.2	13.8	13.9
J. D.	62	M	80.2	Gout	ACTH	100	4	21.8	22.1	22.1	21.0
T. T.*	14	M	35.0	Rheumatoid spondylitis	ACTH	100	39	11.8	12.4	12.5	12.0
P. G.	52	M	73.8	Rheumatoid arthritis	Cortisone†	100	5	—	17.0	17.6	17.0

* Bromide had been administered four weeks previously. † Administered intramuscularly.

and one cortisone. Measurements were made at four, five, seven, and 24 hours both in untreated and ACTH treated patients. Correction for bromide excretion was applied to the 24 hour determination. It was observed that the spaces measured at four, five, seven, and 24 hours were in good agreement (Table I). In most of our data, the five hour, or average of the five and seven hour spaces is given. In patients in whom considerable weight gain as a result of ACTH administration had occurred, the five, seven and 24 hour spaces were measured to establish adequate equilibration.

When a number of estimations of the bromide space were made at approximately weekly intervals, the bromide blank value in some instances rose to levels approximating 50% of the total bromide concentration in the plasma (Table II). In order to correct for the change in the magnitude of high blank values as a result of excretion during determinations of the 24 hour space, the zero time blank value was corrected as follows:

$$\frac{\text{Blank Value}}{\text{24 hr.}} = \frac{\text{Blank Value}}{\text{0 hr.}} \times \frac{[Br^-]\ \text{24 hr.}}{[Br^-]\ \text{5 hr.}}.$$

This correction assumes that administered bromide is completely diffused in five hours, and that fall in the bromide ion concentration after five hours is due completely to excretion. Support for this assumption is presented in Table II.

Total body chloride: Weir and Hastings (10) and Wallace and Brodie (11) have shown that, except in brain and spinal fluid, bromide distributes itself in tissue in proportion to the chloride content, and that the ratio of the concentrations of bromide to chloride is constant in the different tissues of the body. This fact makes it possible to determine total body chloride, at least as an approximation, on the basis of the distribution of bromide. This has been done in man by Dunning, Steele, and Berger (8), and Deane, Ziff, and Smith (12). Total chloride was calculated according to the following formula:

$$\text{Total mEq. } Cl^- = \frac{(\text{mg. } Br^- \text{ administered} - \text{mg. } Br^- \text{ excreted}) \times \text{mEq. } Cl^-/\text{ml. plasma}}{\text{mg. } Br^-/\text{ml. plasma}}.$$

Serum electrolytes: Plasma potassium and serum sodium concentrations were determined on the flame photometer, serum chloride was determined by the method of Wilson and Ball (13) and plasma bicarbonate by the manometric method of Van Slyke and Neill (14). The serum chloride levels include bromide but the latter was less than 2% of the total halide.

Satisfactory eosinophil responses to ACTH (15) were

TABLE II

Bromide spaces measured at five and 24 hour intervals in ACTH treated patients who had previously received bromide

Patient	Age	Sex	Wt.	ACTH dosage	Days	Bromide (*mg. per ml.*)				Bromide space (*liters*)	
						Blank value	5 hr.	24 hr.	Blank value (24 hr.) corr.	5 hr.	24 hr.
	yrs.		*Kg.*	*mg. per day*							
H. C.	17	F	50.7	100	12	.087	.164	.157	.083	15.9	16.2
W. C.	42	F	51.8	120	4	.089	.194	.186	.085	15.1	15.1
W. C.	42	F	58.0	120	18	.159	.224	.209	.148	22.1	21.9
R. F.	14	M	61.2	100	47	.105	.191	.174	.096	17.9	17.7

TABLE III

Repetition of space and body chloride determinations in same individual untreated with ACTH

F. R., Male, 53 (Rheumatoid arthritis)

Determination	Day			
	1	5	10	16
Plasma volume (*liters*)	2.78	—	2.98	2.91
Bromide space* (*liters*)	15.5	16.0	14.8	15.3
Total body Cl^- (*mEq.*)	1,810	1,850	1,720	1,770
Antipyrine space (*liters*)	36.5	35.3	35.6	36.8
Weight (*Kg.*)	59.5	58.6	59.8	60.7

* Five hour space.

noted in all patients treated with this hormone except E. S.[5]

RESULTS

It may be seen from Table I that in untreated as well as in ACTH treated patients who had not received bromide previously, bromide spaces at four, five, seven and 24 hours were essentially equivalent. In Table II are presented measurements of the bromide space in ACTH treated patients who had received bromide in previous experiments and in whom the zero hour blank was corrected for excretion during the 24 hour period as described. The five and 24 hour spaces are in good agreement.

In order to determine the precision of the determinations used, T 1824, antipyrine and bromide spaces were measured four times within a one week period in the same individual, a 46-year-old man with moderately advanced rheumatoid arthritis (Table III). The widest variation in four determinations was 0.2 liter in the T 1824 space, 1.2 liters in the bromide space, 1.5 liters in the antipyrine space, and 130 mEq. in the calculated body chloride.

In Figures 1 to 5 are plotted the changes in bromide space, total body chloride, antipyrine space and weight occurring in individual patients during and after ACTH administration. These are represented as increments above the control values obtained prior to ACTH administration. They have been plotted on a common scale of ordinates so that 1 liter of antipyrine or bromide space, 1 Kg. of weight and 100 mEq. of chloride each have the same dimension. T 1824 volumes are also given.

Effects of ACTH administration to patients (Figures 1–5)

1). F. R., Rheumatoid arthritis, male, age 53

When studied at six and 18 days after the start of ACTH administration, the patient demonstrated (Figure 1) marked expansion of the antipyrine space, bromide space and total body chloride. After 18 days, 8.1 liters of water had been gained, a 22% increase over the original volume. The bromide space increased 8. 9 liters, and the weight 8.9 Kg. It would appear that the increases in body weight and body water were accounted for by the expansion of the bromide space. T 1824 space increased from 2.9 to 4.1 liters during this period, and the hematocrit fell from 38 to 30. The patient, who was free of cardiac disease, demonstrated mild exertional dyspnea and pitting edema of the legs and presacral region at this time. The venous pressure was 200 mm. of water and the right auricular pressure rose to 9 mm. of Hg.[6]

Ten days after ACTH therapy was discontinued, marked decreases of approximately equal magnitude had occurred in all compartments measured. The venous pressure had returned to normal, and there was no edema. Serum electrolyte levels (Na^+, K^+, Cl^-, and HCO_3^-) remained within normal limits during and after ACTH administration (Table IV).

2). G. R., Rheumatoid spondylitis, with peripheral joint involvement, male, age 52

This patient was free of cardiac disease but had 2 + pitting edema of the feet associated with joint involvement of both ankles. As in the preceding patient, after ACTH had been administered for 13 days (Figure 2), there was a 26% increase in

[5] In this patient, the eosinophil count, initially 321, fell to 108 after 14 days and to 21 after 17 days of ACTH administration, remaining reduced during the remainder of the period of hormonal therapy. The pre-treatment 17-ketosteroid excretion was 6.8 mg. and rose to 21 mg. after 17 days of treatment. Serum electrolyte concentrations, Robinson-Power-Kepler excretion test and glucose tolerance tests were within normal limits. There were no clinical evidences of adrenal insufficiency. The response to treatment was somewhat delayed but adequate after the first week of therapy.

[6] Cardiac catheterizations referred to in this paper were done by Drs. Ludwig W. Eichna, Roy Albert, and Warren Smith.

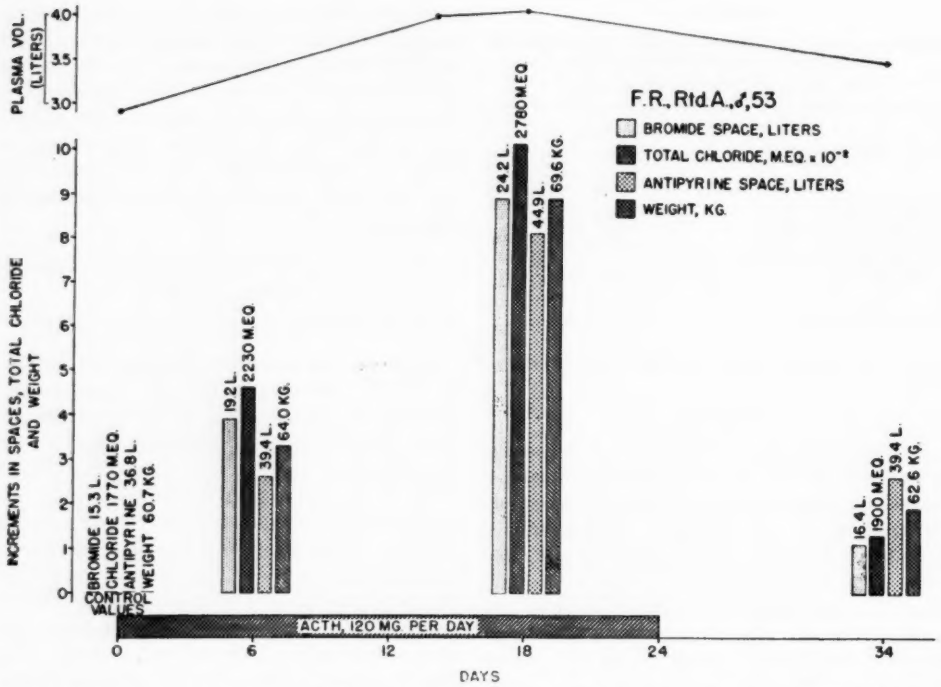

FIG. 1. PATIENT F. R. INCREMENTS IN SPACES, TOTAL CHLORIDE, AND WEIGHT ABOVE CONTROL VALUES OBTAINED PRIOR TO ACTH ADMINISTRATION

total body water, amounting to 8.1 liters, and there were increases of approximately equal magnitude in bromide space and body weight. Here again the weight and water gain were equalled by the expansion of the bromide space. The T 1824 space varied in an opposite direction to the bromide and antipyrine spaces, decreasing from 4.1 to 3.4 liters during the 13 day period. This change was accompanied by a rise in the hematocrit from 28 to 32. Serum K^+ concentration fell from 5.5 to 3.0 mEq. per liter and serum Cl^- from 106 to 96 mEq. Sodium levels remained within normal limits.

3). M. U., Rheumatoid arthritis, female, age 28

During the first eight days of treatment (Figure 3), the patient responded like the preceding ones with significant and approximately equal expansions of the antipyrine space, bromide space, total body chlorides, and body weight. After 19 days of treatment, however, the water and weight increments were significantly greater than the expansion of the bromide space, and after 33 days this divergence was even more marked. Whereas the bromide space had increased only 2.2 liters above the pre-ACTH level, body water had increased 5 liters and the weight 6.5 Kg. Six days following discontinuance of ACTH, the bromide and antipyrine spaces had diminished in volume approximately 1 liter to 1.5 liters. Eight days after ACTH was stopped, her weight had diminished only 1.4 Kg. or 22% of the maximum increment. T 1824 space showed a gradual increase during therapy, going from 2.1 to 2.6 liters in 25 days, and returning to the original value after discontinuance of ACTH. Serum electrolytes remained at normal values throughout. The patient developed no edema nor manifestations of cardiac decompensation.

4). L. G., Rheumatic fever, male, age 40

This patient had a history of rheumatic polyarthritis with fever and a 20 lb. weight loss during a

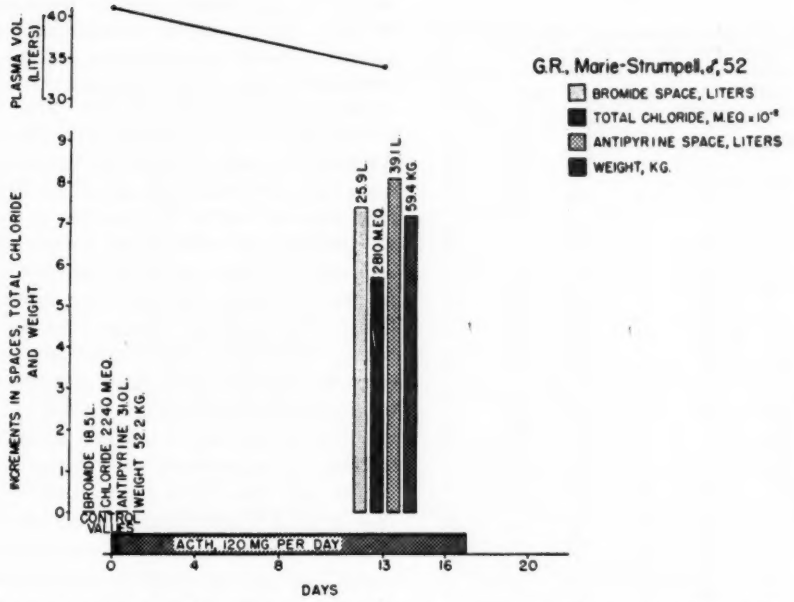

FIG. 2. PATIENT G. R. INCREMENTS IN SPACES, TOTAL CHLORIDE, AND WEIGHT ABOVE CONTROL VALUES

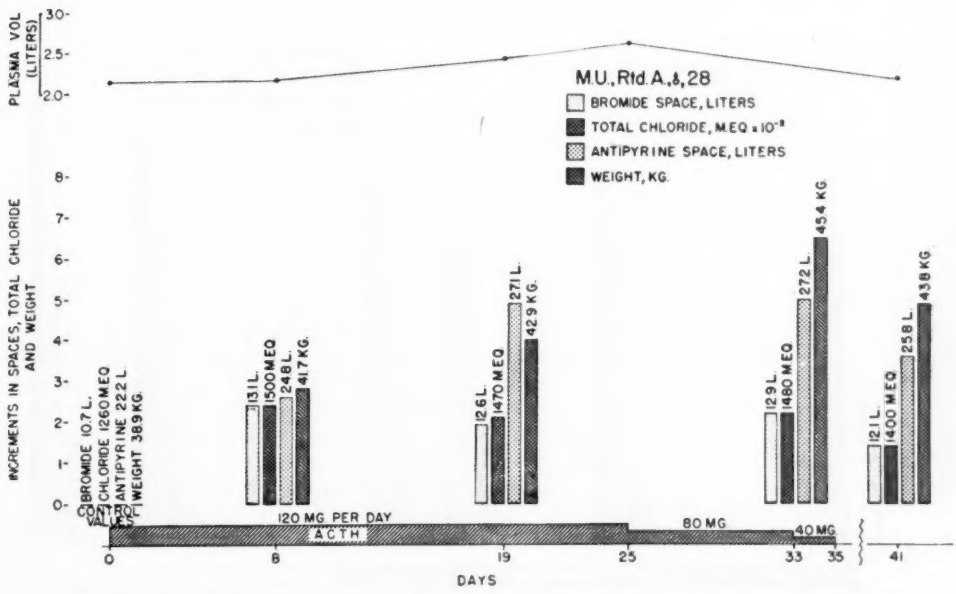

FIG. 3. PATIENT M. U. INCREMENTS IN SPACES, TOTAL CHLORIDE, AND WEIGHT ABOVE CONTROL VALUES

TABLE IV

Serum electrolyte concentrations and hematocrits before, during and after administration of ACTH

Patient	Day	Na^+	K^+	Cl^-	HCO_3^-	Hct.
		mEq./L.	*mEq./L.*	*mEq./L.*	*mEq./L.*	
F. R.	Control	139	3.9	102	24.1	38
	6	143	3.3	102	25.2	36
	18	138	4.2	100	26.2	30
	34	135	4.6	104	22.5	32
G. R.	Control	141	5.5	106	—	28
	13	134	3.0	96	—	36
M. U.	Control	140	4.6	104	22.5	31
	8	141	4.1	102	22.5	32
	19	139	3.4	103	29.7	33
	25	134	4.3	100	26.2	34
	33	141	3.5	99	24.7	34
	41	136	3.8	102	24.4	34
L. G.	Control	140	4.8	102	19.8	37
	11	137	4.5	101	26.1	38
	19	134	3.5	101	26.1	37
	21	138	3.5	103	26.8	37
	33	137	3.9	105	23.6	37
	41	137	3.9	104	23.4	37
E. S.	Control	135	5.1	98	—	31
	6	140	3.4	100	26.1	33
	10	141	3.3	95	25.2	35
	25	134	3.7	94	24.7	37
	35	136	3.1	97	28.3	36

period of one month prior to treatment with ACTH. There was no evidence of rheumatic carditis. The disease was active and the patient febrile when therapy was instituted. After 11 days of ACTH administration, he had gained 7.5 Kg. and 8.2 liters of water (Figure 4). This was accompanied by only a negligible increase in bromide space and total body chloride. The latter two quantities increased somewhat as treatment was continued, but after 21 days the bromide space was only 1.5 liters above the pre-ACTH value, whereas the antipyrine space had increased 6.4 liters. The weight had increased 8.4 Kg. at this time. Sixteen days after ACTH had been discontinued, the total body water still remained at its expanded volume and the patient had gained an additional 2.2 Kg. of weight. Bromide space had shown a comparatively small decrease. T 1824 volume rose from 3.5 to 4.2 liters during the 21 days of ACTH administration and did not fall after this was discontinued. Hematocrit was unchanged and serum electrolyte concentrations remained within normal limits throughout. Cardiac

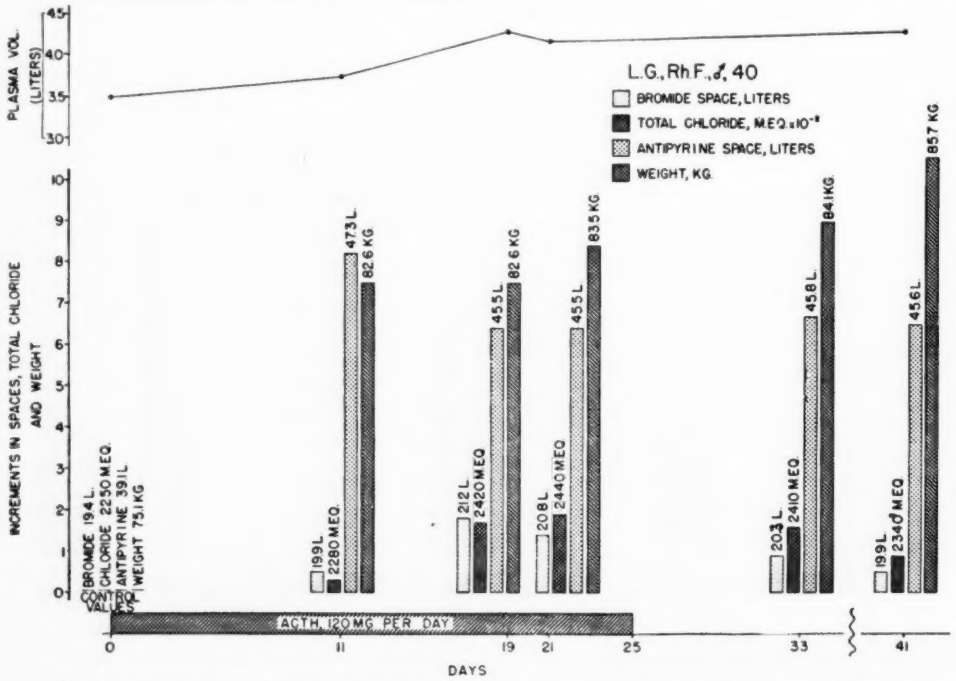

FIG. 4. PATIENT L. G. INCREMENTS IN SPACES, TOTAL CHLORIDE, AND WEIGHT ABOVE CONTROL VALUES

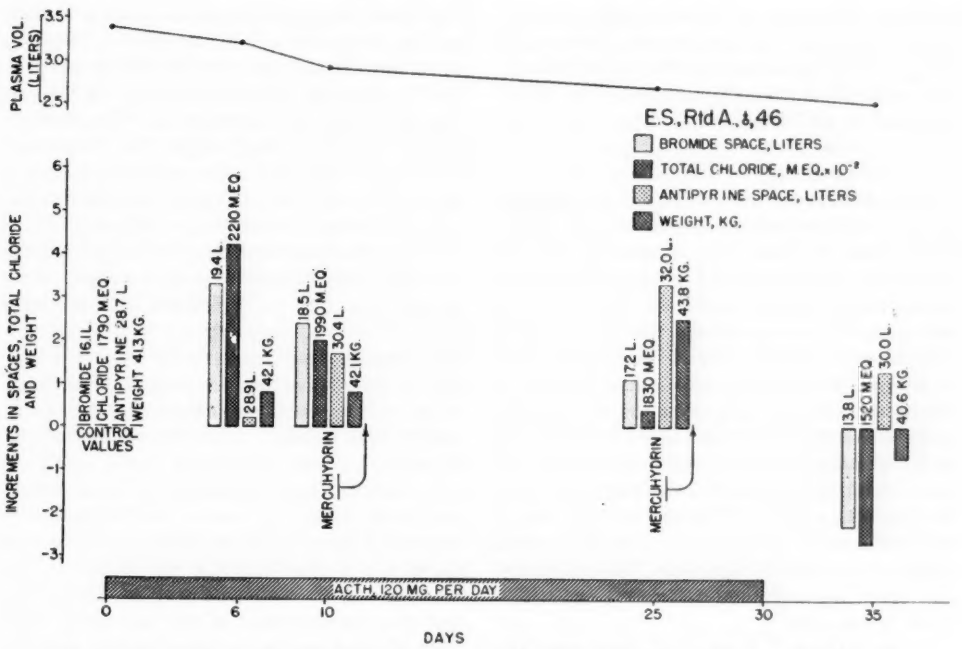

FIG. 5. PATIENT E. S. INCREMENTS IN SPACES, TOTAL CHLORIDE, AND WEIGHT ABOVE CONTROL VALUES

Mercuhydrin (2 ml.) was injected intramuscularly following the space determinations on the 10th and 25th days of ACTH administration.

catheterization was done on the 19th day of therapy and the results indicated no abnormality of cardiac function. The patient had no symptoms or signs of diminished cardiac reserve.

5). E. S., Rheumatoid arthritis, female, age 46

This patient had advanced rheumatoid arthritis and evidence of inactive chronic heart disease, consisting of cardiac enlargement and insufficiency of the mitral valve. There were no signs of failure. Six days after the administration of ACTH, she had retained 420 mEq. of chloride (Figure 5). This was accompanied by an increase of 3.3 liters of the bromide space, but only by a negligible increase in body water and body weight. There was at this time dyspnea at rest, mild pitting edema of the ankles, and a venous pressure of 160 mm. of water. Following space studies, a mercurial diuretic was injected to relieve the symptoms of failure. As ACTH was continued, there was a diminution in the bromide space and body chloride. This was accompanied, however, by a rise in total body water and weight. At 25 days, the bromide space and total chloride had returned almost to control levels whereas total body water had increased 3.3 liters above the pre-ACTH value. At this time the patient was again complaining of dyspnea and orthopnea, the liver was enlarged, there was edema of the lower extremities and the venous pressure was 160 mm. of water. Blood circulation times were within normal limits. Following space studies, a mercurial diuretic was again given. Five days after ACTH was discontinued, there was a marked diminution of bromide space and total chloride to significantly less than pre-ACTH levels. This was accompanied by smaller, though significant, losses in body weight and water.

T 1824 space showed a steady fall throughout therapy, diminishing from a pre-treatment value of 3.4 liters to a volume of 2.7 liters after 25 days of ACTH, and remaining at approximately this level, five days after the hormone was discontinued. The hematocrit rose from 31 to 37 and the hemoglobin from 10 to 11.5 grams in the course of the 25 day period of ACTH administration. Serum Na^+

remained within normal limits throughout the period of observation; K^+ concentration fell from 5.1 mEq. to 3.7 mEq. per liter at 25 days; serum chloride varied from 98 to 94 mEq.; and the HCO_3^- level rose to 29.7 mEq. at this time.

COMMENT

It has been demonstrated (10, 11) that bromide is distributed similarly to chloride in body tissues. The volume of distribution of bromide, like that of chloride, thiocyanate, and sodium, is larger than that of inulin and sucrose (7, 12, 16), and it has been suggested, for this reason, that approximately 30% of body chloride (12) is intracellular. If it is accepted that chloride exists intracellularly in significant amounts, expansion of the bromide space induced by ACTH may reflect in part change in intracellular as well as extracellular fluid volume. Antipyrine space (4a) was utilized to measure total body water. Evidence that the volume of distribution of antipyrine is equivalent to total water is the observed agreement between antipyrine space and deuterium oxide (4a) and tritium oxide spaces (4b).

In two patients, F. R. and G. R., there were large increases in total body water, which paralleled quite closely weight gain and expansion of the bromide space. In these patients, it would appear that the increase in body water was associated largely with expansion of the chloride compartment. This is consistent with the fact that both patients developed increasing edema and that in one (F. R.) the venous pressure rose to abnormal levels. In M. U. and L. G., however, the expansion of the chloride compartment did not keep pace with the increase in total body water. This could in part be due to the tendency to loss of chloride with alkalosis which is known to occur in patients with hyperadrenalcorticalism (1), but significant abnormality of serum electrolyte concentrations was not noted in either individual. It is likely that the water increments in M. U. and L. G. represent largely expansion of the intracellular compartment, which is ordinarily associated predominantly with retention of anions other than chloride. This could in great part be due to repair of atrophic muscle tissue, 83% of which, according to Hastings and Eichelberger (17), consists of intracellular phase of which 72% is water. The history of marked weight loss prior to therapy and the absence of a water diuresis exhibited by L. G., and similarly the small diuresis observed in M. U. following discontinuance of ACTH, are consistent with this explanation. The relatively greater increase in body weight than body water observed in these two cases at the termination of therapy may be explained by the deposition of significant amounts of body fat and tissue solids.

E. S., who had advanced rheumatoid arthritis and also heart disease, showed a pattern of response to ACTH which differed from the other four. There was early increase in body chloride and bromide space, without concurrent expansion of body water or body weight. Simultaneously there appeared symptoms and signs of congestive heart failure. A somewhat similar phenomenon has been reported by Levitt and Bader (2), and has been attributed by these authors to a shift of salt and water extracellularly. In the case of chloride, this would have to arise from stores which previously did not exchange with bromide. With continuation of ACTH therapy and the administration of two mercurial injections, there was a loss of body chloride and, five days after discontinuing ACTH, total body chloride and bromide space were well below pretreatment values.

The plasma volume rose with ACTH administration in three patients and fell in two. In four out of five, changes in plasma volume corresponded fairly well with changes in the bromide space. In G. R., plasma volume fell as bromide space rose. Jager, Brown and Nickerson (18a) noted an increase in plasma volume in three patients out of five receiving ACTH; in the other two patients there was a large and small decrease, respectively.[7] The authors suggest that shifts of protein into and out of the vascular compartment, induced by ACTH, might play a role in altering the plasma volume. Repair of anemia may be a potent factor in decreasing plasma volume. Thus, in G. R. there was a rise in hemoglobin from 9 to 11 grams during ACTH therapy, and the hematocrit rose

[7] Copeman and co-workers (18b) have recently observed a fall of plasma volume in all of three patients studied, who were being treated with cortisone; this occurred in spite of the fact that all showed some degree of fluid retention.

from 28 to 32. The possibility of alteration in capillary permeability to T 1824 as a result of adrenal cortical stimulation should also be considered as a factor affecting the measured blood volume. Change in capillary permeability to T 1824, in rats treated with cortisone (19), was not observed, however, when hyaluronidase was not injected simultaneously.

The suggestion has been made by McMaster and Parsons (20) on the basis of microscopic observations of the manner of interstitial movement of vitally injected dyes that there is no free fluid, in the ordinary sense, in the ground substance of normal connective tissue. These authors could not identify the presence of freely movable fluid in the interstitial space. The demonstration of permeability changes in tissue treated locally with hyaluronidase and cortisone (21, 22) raises the question as to whether the extent of diffusion of materials through the ground substance, with which the water may be bound, might be altered sufficiently by hormonal therapy to affect the measured total body water. Increase in antipyrine space without at least an equivalent gain in weight was not observed, however, in the present studies. Nevertheless, the possibility of diminution in antipyrine space because of decreased permeability, such as occurs with prolonged local treatment with cortisone (23), cannot be ruled out.

Assays of the lot of ACTH used,[8] done in the laboratory of Dr. E. P. Ralli, indicated the presence of 20 milliunits of antidiuretic (ADH) activity (24) per mg. of ACTH (Armour Standard LA-1-A). Thus each dose of 25 mg. contained 500 milliunits of ADH activity. It would appear unlikely that this dosage in aqueous solution, given at six hour intervals, would by itself bring about a sustained retention of water. Lloyd, Harris, and Lobotsky (25) observed no gain in weight in two normal individuals receiving daily injections of pitressin tannate in oil. Robinson and Farr (26) administered 30 units of pitressin per day to humans and, though there was an early rise in urinary ADH, it fell rapidly to normal in a few days. The combined effect of ACTH and pitressin on water and salt balance is a subject about which there is as yet little information (27).

[8] J-16709 R, Armour and Co.

It would appear from the present work, that the amount of water retention, and the manner in which this is retained in response to ACTH varies in different individuals, being in some predominantly extracellular and in others intracellular. Variability of response to adrenal cortical hormones under differing physiological conditions has been noted commonly. Cortisone may, under different circumstances, cause either water retention or water diuresis (27). Impairment of glucose tolerance may occur in some patients after a brief period of cortisone administration, and in others no impairment is demonstrable even after prolonged administration (28). Nitrogen balance may be either positive or negative according to existing tissue requirements (29). It may be that the manner in which water is retained in response to ACTH is determined among other things by the tissue needs of the subject. Thus, water retention appeared to be intracellular in L. G., for example, in whom body solids increased, and extracellular in F. R. in whom body solids did not increase.

SUMMARY

Simultaneous determinations of T 1824, antipyrine and bromide spaces were made before, during and after ACTH therapy in five patients with rheumatic diseases. Total body chloride was calculated at the same time. The following observations were made:

1) On administration of ACTH, marked increases in body water were consistently observed in all cases. *2*) In two patients who developed increasing edema on administration of ACTH, one of whom demonstrated elevation of venous pressure, the expansion of antipyrine space and gain in body weight were similar in magnitude to increase in the bromide space, indicating retention of water predominantly in the extracellular compartment. *3*) In two patients in whom edema or signs of venous congestion were not evident, increase in antipyrine space and body weight exceeded the increment in bromide space. The water increments in these patients appeared to be due mainly to retention of intracellular water associated with gain in tissue weight. *4*) In one patient there was an initial increase in bromide space with retention of chloride, which later diminished in

spite of an increase in total body water. *5)* T 1824 space rose in three patients and fell in two during administration of ACTH. It varied directly with the bromide space in four patients out of five. *6)* The manner of water retention by different individuals in response to ACTH is variable.

ACKNOWLEDGMENTS

We are grateful to Dr. Denys Ford for his cooperation in this investigation, and to Mrs. Jennie Shatton, Mr. Harold Kadin, and Mr. Martin Tanner for their technical assistance.

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THE ALVEOLAR–ARTERIAL OXYGEN PRESSURE GRADIENT IN MITRAL STENOSIS [1]

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In 1931 zu Jeddeloh (1) reported his observations on the alterations in the alveolar walls in chronic passive congestion. Parker and Weiss (2) in 1936 sharply focused attention upon these changes, particularly in patients with "tight" mitral stenosis. These authors demonstrated the dilatation of the capillaries and the thickening of the alveolar walls that occurred secondary to chronic engorgement of the pulmonary capillary bed. Larrabee, Parker, and Edwards (3) confirmed these findings in recent studies.

These structural changes at the level of the alveolar-capillary membrane might be expected to interfere with the diffusion of oxygen across this membrane. Decreased oxygen saturation of the peripheral arterial blood has been frequently observed in patients with mitral stenosis. This fact would suggest the possibility that the blood leaving a significant fraction of the pulmonary capillaries had not been fully oxygenated. Thus, it was suggested that a physiologic counterpart to the known pathologic changes might be present and effect a change in the normal alveolar-capillary oxygen tension gradient.

The present report deals with the determination of this gradient in patients with "tight" mitral stenosis.

MATERIAL AND METHODS

The normal values were determined from studies of 20 medical students in good health. There were three females and 17 males with an average age of 26.1 years. Observations were made on 18 patients with rheumatic heart disease and mitral stenosis. The average age of these patients was 31.7 years, with the sexes equally divided. In two patients organic tricuspid regurgitation was also present, and, in a third, aortic stenosis was present. The degree of their incapacitation was only mild to moderate. Nine of the patients were classified in group II, and nine in group III by the criteria of the New York Heart Association (4).

Arterial blood was obtained through an indwelling needle in the brachial artery with the patient supine. At least five minutes were allowed after placement of the needle before withdrawing blood. Oxygen content and capacity and carbon dioxide content were determined in duplicate using the Van Slyke-Neill (5) manometric method. The arterial blood for oxygen tension determination was withdrawn simultaneously with the collection of the expired air for a one and one-half minute period. The partial pressures of oxygen and carbon dioxide in the arterial blood were determined by the direct technique of Riley, Proemmel, and Franke (6). The alveolar partial pressure of oxygen was calculated using the alveolar equation in the form presented by Pappenheimer and his associates (7), substituting arterial PCO_2 for alveolar PCO_2. The alveolar-arterial oxygen pressure gradient was then calculated. Venous cardiac catheterization was performed in all patients in the mitral group. Procedures were carried out at average ambient temperature of 23.5° C. and a barometric pressure of 623 mm. Hg. These determinations were then repeated with exercise, using a bicycle arrangement with the patient in a upine position. The amount of exercise was that sufficient to produce subjective fatigue.

RESULTS

The pertinent data from the normal subjects are shown in Table I and from the patients with mitral stenosis in Table II. A significant difference existed between the mean values for resting arterial oxygen in the tension in the two groups. The mean value for the normal subjects was 74.8 mm. Hg and for the patients with mitral stenosis 66.3 mm. Hg. No significant difference existed between the mean values of alveolar oxygen tension in the two groups at rest. The resting alveolar-arterial oxygen tension gradient was significantly higher in the mitral stenosis group than in the normal group; the levels being 11.6 mm. Hg and 4.8 mm. Hg, respectively.

The arterial oxygen tension during exercise was not significantly altered from the resting val-

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[2] Public Health Service Postdoctorate Research Fellow of the National Heart Institute.

TABLE I

The oxygen tension values in the normal subjects

Subject	Arterial oxygen tension (*mm. Hg*)		Alveolar oxygen tension (*mm. Hg*)		Alveolar-arterial oxygen tension gradient (*mm. Hg*)	
	Rest	Exercise	Rest	Exercise	Rest	Exercise
C. B., 28 F	76.4	87.4	74.0	95.5	−2.4	8.1
G. B., 27 M	69.8	83.2	70.9	88.3	1.1	5.1
W. M., 22 M	79.3	81.5	78.0	86.1	−1.3	4.6
V. B., 27 M	84.1	71.4	91.9	82.6	7.8	11.2
D. O., 20 F	74.9	73.8	83.5	87.3	8.6	13.5
M. P., 27 F	75.4	77.4	83.8	86.1	8.4	8.7
D. T., 23 M	72.2	72.7	73.7	83.4	1.5	10.7
J. H., 26 M	75.1	77.4	82.5	85.7	7.4	8.3
R. I., 34 M	74.1	76.0	79.9	87.1	5.8	11.1
K. C., 24 M	68.7	72.8	74.9	83.7	6.2	10.9
T. H., 24 M	71.3	68.7	77.8	77.5	6.5	8.8
G. C., 24 M	70.2	75.9	68.7	82.9	−1.5	7.0
T. B., 24 M	71.8	71.6	67.9	86.3	−3.9	14.7
W. Z., 24 M	71.2	72.5	73.6	78.7	2.4	6.2
M. B., 27 F	69.4	67.8	77.2	89.3	7.8	21.5
T. A., 25 M	70.0	71.8	72.7	83.4	2.7	11.6
T. H., 27 M	85.7	80.2	95.3	85.6	9.6	5.4
G. W., 26 M	85.1	80.8	94.2	98.8	9.1	18.0
S. K., 40 M	70.0	67.2	77.0	88.6	7.0	21.4
J. N., 24 M	81.8	75.4	94.5	82.3	12.7	6.9
Mean	74.8	75.3	79.6	86.0	4.8	10.7
Standard error of the mean	±1.27	±1.23	±1.97	±1.13	±1.06	±1.15

ues in either group. The alveolar oxygen tension level, however, rose to 86.0 mm. Hg in the normal group and to 83.9 mm. Hg in the group with mitral stenosis. The alveolar-arterial oxygen tension gradient on exercise was thereby increased to 10.7 mm. Hg for the normal subjects and to 15.5 mm. Hg in the group with mitral stenosis.

The mean arterial oxygen saturation at rest was 92.1 per cent in the patients with mitral stenosis and 94.1 per cent in the normal subjects.

The resting minute ventilation per square meter of body surface was 3.31 liters in the normal group and 4.12 liters in the mitral stenosis group. The mean arterio-venous oxygen difference in the mitral stenosis group was 5.69 volumes per cent at rest and 9.30 volumes per cent on exercise. The mean cardiac index in the patients with mitral stenosis was 2.74 liters at rest and 3.53 liters on exercise.

TABLE II

The oxygen tension values in the patients with mitral stenosis

Subject	Arterial oxygen tension (*mm. Hg*)		Alveolar-oxygen tension (*mm. Hg*)		Alveolar-arterial oxygen tension gradient (*mm. Hg*)	
	Rest	Exercise	Rest	Exercise	Rest	Exercise
H. M., 38 M	65.6	74.2	84.3	86.4	18.7	12.2
L. S., 45 F	52.3	67.3	61.9	76.7	9.6	9.4
B. M., 40 F	70.0	71.5	84.0	78.7	14.0	7.2
S. C., 38 M	62.0	68.2	71.9	89.5	9.9	21.3
R. M., 18 M	74.5	75.9	85.5	95.7	11.0	19.8
A. S., 43 F	59.1	69.1	73.0	83.3	13.9	14.2
M. B., 37 F	70.3	55.3	84.8	77.2	14.5	21.9
E. V., 49 F	75.2	77.1	76.3	88.6	1.1	11.5
L. M., 30 M	71.4		82.3		10.9	
M. T., 21 M	63.0	58.5	79.4	75.1	16.4	16.6
C. R., 39 F	72.2	71.0	77.3	83.0	5.1	12.0
A. H., 29 F	57.5	60.1	80.8	79.8	23.3	19.7
J. A., 13 M	60.5	56.7	61.0	60.8	0.5	4.1
S. M., 31 M	60.8	64.9	73.2	78.0	12.4	13.1
W. N., 24 M	67.0	75.9	68.8	95.9	1.8	20.0
L. S., 12 M	75.3	70.9	95.5	88.0	20.2	17.1
A. C., 29 F	73.5	79.7	83.9	100.0	10.4	20.3
M. E., 35 F	63.0	66.7	78.3	89.9	15.3	23.2
Mean	66.3	68.4	77.9	83.9	11.6	15.5
Standard error of the mean	±1.67	±1.84	±2.10	±2.38	±1.56	±1.40

DISCUSSION

There was a significant reduction in the arterial blood oxygen tension in the group with mitral stenosis as compared to the normal group, while the alveolar oxygen tension revealed no significant difference between the two groups. The resulting alveolar-arterial oxygen pressure gradient was thus significantly greater in the mitral group due to the lower arterial blood oxygen tension in this group.

Since on the average the alveolar oxygen tensions were very nearly the same in the normal subjects and in the patients with mitral stenosis, there are two main factors to be considered in an evaluation of the lowered arterial oxygen tension in the patients with mitral stenosis. These are venous admixture, in the expanded sense defined by Riley and Cournand (8), and alveolar-capillary oxygen diffusion. There is some admixture of poorly oxygenated blood to the well oxygenated blood leaving the alveolar capillaries even in normal persons. The principal channels through which this takes place are the bronchial radicles draining into the pulmonary veins, the myocardial venous channels draining into the left heart, and the pulmonary capillaries that are perfusing imperfectly aerated alveoli. The oxygen content of the mixed venous blood was lower in the patients with mitral stenosis than in normal subjects and on exercise there was a considerable further decrease in the oxygen content of the mixed venous blood. This would tend to accentuate the normal effect of the admixture of venous blood upon lowering the arterial oxygen tension. This mechanism has been proposed by Ryan and Hickam (9) as an explanation of the lowered arterial oxygen tension and increased alveolar-arterial oxygen pressure gradient in patients with anemia.

Sample calculations using charts prepared by Riley, Cournand, and Donald (10) would indicate that marked impairment of diffusion beyond the realm of probability would have to be present in order to influence significantly the alveolar-capillary difference. The level of alveolar oxygen tension was so high in these patients that abnormalities of diffusion, if present, were not manifested by appreciable contributions to the alveolar-arterial oxygen pressure gradient. This does not necessarily indicate that there is no impairment of diffusion in patients with mitral stenosis as the circumstances under which this study was performed were not such as to determine this factor.

To determine diffusion impairment at the alveolar-capillary membrane level studies as presented in this report must be performed at two levels of oxygenation as described by Riley, Cournand, and Donald (10). The two levels of oxygenation are designed to effect a definite and significant change in the relative magnitude of the two components of the alveolar-arterial oxygen pressure gradient; namely, diffusion and venous admixture. Such studies are now in progress in this laboratory.

SUMMARY AND CONCLUSIONS

1. The arterial oxygen tension, alveolar oxygen tension and the resulting alveolar-arterial oxygen pressure gradient have been determined in 20 normal subjects and in 18 patients with mitral stenosis at rest and with exercise.

2. At rest the mitral group had a normal alveolar oxygen tension, a significantly decreased arterial oxygen tension, and a resulting increased alveolar-arterial oxygen pressure gradient as compared with the normal control group.

3. On exercise the alveolar oxygen tension rose in both groups and the arterial oxygen tension remained essentially unchanged in both groups.

4. The increased alveolar-arterial oxygen pressure gradient in patients with mitral stenosis, as determined under the conditions of the present study, may be explained on the basis of increased venous admixture.

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BLOOD LEVELS OF 17-HYDROXYCORTICOSTEROIDS FOLLOWING THE ADMINISTRATION OF ADRENAL STEROIDS AND THEIR RELATION TO LEVELS OF CIRCULATING LEUKOCYTES [1]

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INTRODUCTION

In 1944 Dougherty and White (1) reported the occurrence of neutrophilia and lymphopenia following the administration of ACTH or adrenal cortical extracts to mice. In 1948 these findings were confirmed in man by Hills, Forsham and Finch (2), who noted, in addition, a pronounced fall in eosinophils. These changes in the blood have been used widely as an index of adrenal cortical activity. With the development of a method for the determination of 17-hydroxycorticosteroids in small quantities of blood or plasma (3) it has become possible to determine the relationship of the changes in blood steroid levels to the changes in leukocytes.

In order to investigate this relationship, standard doses of various corticosteroids were administered to normal individuals and the alterations in blood 17-hydroxycorticosteroids and in leukocytes were observed at intervals thereafter.

METHODS

Compound E (17-hydroxy-11-dehydrocorticosterone), compound F (17-hydroxycorticosterone), and compound S (17-hydroxy-11-desoxycorticosterone) were administered as the free alcohols and as the acetate esters. All subjects used were normal young adult males who were fasting at the time the steroids were given, and for at least three hours thereafter. When administered orally, the compounds were mixed with fruit juice, with the exception of compound E acetate, which was given as tablets to two subjects (C. F. and T. O.). When administered intramuscularly, the compounds were in the form of a commercially available saline suspension except as indicated in the tables. Generally, the steroids were administered between 8:00 and 9:00 a.m. Blood was drawn for the steroid and blood cell determinations immediately prior to administration, and at one, four, eight, and 24 hours thereafter. These intervals were selected after preliminary studies in which samples were drawn at one-half hour intervals had demonstrated that the serum steroid peak occurred at approximately one hour and the previous observation of maximum cellular effects at approximately four hours was confirmed. Approximately 30 ml. of blood were drawn at each sampling. Heparin was used as an anticoagulant for the steroid determination and mixed oxalate for the cellular studies.

Determination of 17-hydroxycorticosteroids was carried out by the method of Nelson and Samuels (3). Ten milliliter samples of plasma were generally used for the determination. The Florisil chromatographic column was employed for purification and the micromodification of the color reaction of Porter and Silber was used for quantitation (4). Compounds E, F, and S give this reaction. All levels of 17-hydroxycorticosteroids given are expressed in terms of μgm. per 100 ml. of plasma.

Eosinophil counts were performed in duplicate by the method of Randolph (5). Total leukocyte counts were done in duplicate by routine methods and the average of the two counts was used. Absolute neutrophil and lymphocyte counts were calculated from the total leukocyte and differential counts. The latter were made from cover-slip blood films stained with Wright's stain, on which 500 cells were enumerated.

RESULTS

Figure 1 shows a representative response of leukocytes and plasma 17-hydroxycorticosteroid levels following the administration of 200 mgm. of compound F acetate orally. The data on all seven subjects who received this dose of compound F acetate are shown in Table I. There was in each instance a marked rise in plasma levels of corticosteroids which reached a peak approxi-

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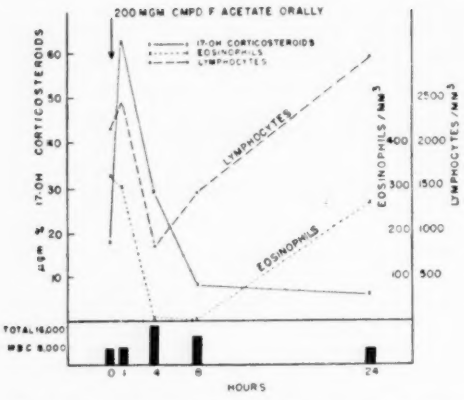

FIG. 1. THE EFFECTS OF 200 MGM. OF F ACETATE GIVEN ORALLY ON BLOOD 17-HYDROXYSTEROID LEVELS AND CIRCULATING WHITE BLOOD CELL LEVELS

Note that although the peak of corticosteroid level occurred at one hour, only negligible changes had appeared in the cellular elements at that time. At four hours when the steroid level had fallen sharply, the maximum effect on cellular elements made its appearance.

mately one hour after the steroid was given. The level then fell rapidly and returned to approximately normal at the end of eight hours. Although the highest concentration of steroids was reached at about one hour, the maximum change in leukocytes was not seen until four or eight hours after ingestion of the compound. In every instance a decrease in lymphocytes and eosinophils occurred as well as a pronounced rise in neutrophils; the result was a rise of the total white count.

A single subject was given oral doses of compound F acetate ranging from 12.5 to 200 mgm. in order to determine the relation of dose to the response obtained. The experiments were carried out at approximately five-day intervals to minimize the effect of repeated dosage in the same subject. The results obtained are illustrated in Figure 2. When the levels at four hours are considered, it appears that the magnitude of the response in 17-hydroxycorticosteroids is progressively greater and more prolonged as the dosage of compound F is increased. Eosinophil levels fell at all dosage levels. With each increase in compound F a greater fall in eosinophils occurred until doses of 100 to 200 mgm. were given. With the latter doses, the eosinophils remained at very low levels for longer periods of time. Lymphocytes decreased with doses of 25 mgm. or more, the amount and duration of the decrease being roughly correlated with the dosage. Increases in neutrophils were also seen at each dose level but this was not correlated with the amount of compound given.

The effects of single injections of 200 mgm. of compound F acetate given intramuscularly to four individuals are shown in Table II. There was no rise in blood levels of corticosteroids and no significant effect upon the leukocytes.

TABLE I

Administration of 17-hydroxycorticosterone (Compound F) acetate, 200 mgm. orally

Subject	Time in hours	17-OH steroid levels, γ/100 ml. of plasma	WBC	PMN	Lymph	EOS
				Absolute number per mm.³		
M. C.	0	8.0	12,600	8,580	3,230	269
	½	48.0				
	1	124.0	12,350	8,590	2,940	288
	2		16,900	13,950	1,900	294
	4	70.0	18,700	16,200	1,980	19
	6		14,050	12,300	1,460	37
	8		13,200	11,100	1,580	19
	24	<10.0	8,800	4,950	3,130	225
A. D.	0	8.4	5,080	2,733	2,022	88
	1	107.2	6,650	4,043	2,141	125
	4	61.2	10,450	9,446	836	25
	8	5.4	9,850	7,900	1,694	6
	24		6,250	3,450	2,350	76
O. H.	0	18.2	7,100	4,075	2,144	325
	1	63.2	7,600	4,530	2,446	300
	4	29.3	19,050	17,640	838	6
	8	7.7	13,600	11,750	1,442	0
	24	6.2	7,300	3,781	2,964	263
D. T.	0	8.0	8,950	4,887	3,204	263
	1	80.2	7,650	5,110	1,943	138
	4	60.1	8,900	7,191	1,549	25
	8	13.7	12,500	11,325	925	6
	24	11.4	7,700	4,004	3,234	263
J. E.	0	10.7	5,000	2,830	1,730	181
	1	63.6	5,350	2,857	1,937	194
	4	50.0	5,900	4,838	896	19
	8		10,000	9,020	780	0
	24	2.4	8,400	3,914	3,814	238
J. B.	0	11.0	4,400	2,692	2,659	100
	1	87.0	4,900	3,386	2,789	113
	4	58.0	6,700	6,372	1,057	13
	8	9.0	7,950	5,624	3,572	6
	24	15.0	5,550	2,950	2,549	138
T. O.	0	11.0	6,160	4,151	1,602	150
	1	98.0	7,400	5,239	1,746	100
	4	50.0	18,350	16,698	1,431	19
	8	21.3	12,675	10,977	1,369	6
	24	20.0	13,400	8,576	4,100	106

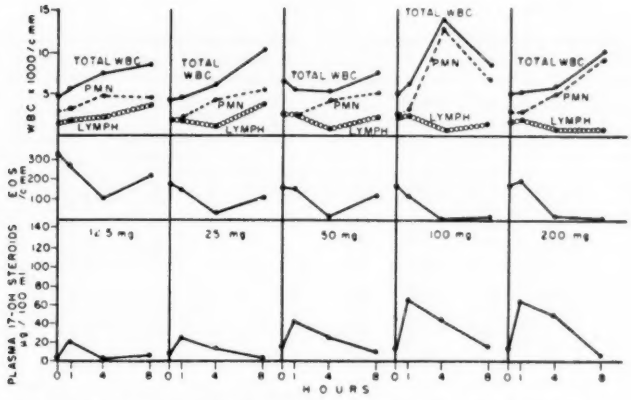

FIG. 2. THE EFFECT OF VARYING DOSES OF COMPOUND F ACETATE GIVEN ORALLY TO A SINGLE SUBJECT

The effects of 200 mgm. of cortisone acetate given orally to five subjects are tabulated in Table III. The effects were similar to those seen following compound F acetate given orally, but were less marked. The rise in steroid levels was in most cases not so great and was less prolonged; the same is true of the changes in leukocytes. In Table IV are shown data on four subjects given cortisone acetate intramuscularly. The results are less consistent than those shown previously. In one subject (C. C.), there was a marked rise

TABLE II

Administration of 200 mgm. 17-hydroxycorticosterone (Compound F) acetate I. M.

Subject	Time in hours	17-OH steroid levels, γ/100 ml. of plasma	WBC	PMN	Lymph	EOS
				Absolute number per mm.³		
T. O.	0	19	7,850	4,773	2,685	113
	1	19.2	5,900	3,741	1,841	154
	4	8.4	8,250	5,659	2,145	156
	8	6	10,900	8,349	2,180	175
	24	4	7,350	4,293	2,675	81
M. C.	0	4.6	13,150	7,601	4,576	294
	1	0	13,200	8,791	3,379	356
	4	8.8	12,900	8,514	3,638	400
	8	8.0	11,750	7,755	3,219	250
	24	1.0	16,600	11,919	3,386	331
J. B.	0	19	5,500	2,387	2,640	106
	1	22.6	5,900	2,773	2,669	81
	4	14.4	7,500	3,960	3,090	81
	8	12	8,650	5,916	2,301	75
	24	16.8	5,050	2,545	2,122	125
A. D.	0	8.0	6,400	4,250	1,920	100
	½	10.2				
	1		6,700	4,680	1,860	69
	2		7,800	5,410	1,860	150
	4	4.2	8,350	5,650	1,950	75
	6		8,550	6,550	1,408	100
	8	5.4	9,150	6,860	1,755	50

TABLE III

Administration of 200 mgm. 17-hydroxy-11-dehydrocorticosterone (Compound E) acetate orally

Subject	Time in hours	17-OH steroid levels, γ/100 ml. of plasma	WBC	PMN	Lymph	EOS
				Absolute number per mm.³		
O. H.	0	14	8,700	4,768	2,871	344
	1	25.2	9,500	5,833	2,907	288
	4	11.7	10,100	8,040	1,575	25
	8	9.7	10,200	6,936	2,754	90
	24	8.0	8,800	4,453	3,802	281
D. T.	0	7.8	6,200	3,174	2,554	125
	1	46.0	6,600	3,986	1,848	225
	4	20.0	6,950	5,463	1,251	25
	8	4.0	9,000	5,580	2,898	69
	24	6.0	6,800	2,679	3,754	194
C. C.	0	3.0	8,500	4,641	3,043	238
	1	124.0	9,100	5,187	3,130	275
	4	21.0	12,600	10,483	1,789	13
	8	7.0	12,400	9,796	2,306	56
	24	4.0	8,450	4,326	3,566	269
C. F.	0	11.0	10,800	6,523	3,370	394
	1	65.0	11,000	6,490	3,564	369
	4	16.0	9,500	7,163	1,995	62
	8	5.0	7,850	4,569	2,747	131
	24	10.0	7,800	3,479	3,526	356
T. O.	0	9.6	7,500	4,440	2,775	81
	1	50.0	6,850	4,370	2,206	100
	4	33.0	6,050	4,985	920	38
	8	1.3	12,750	10,404	1,912	44
	24	4.0	9,000	5,760	2,664	138

TABLE IV

Administration of 200 mgm. of 17-hydroxy-11-dehydrocorticosterone (Compound E) acetate I. M.

Subject	Time in hours	17-OH steroid levels, γ/100 ml. of plasma	WBC	PMN	Lymph	EOS
				Absolute number per mm.³		
C. C.	0	16.8	6,550	3,733	2,306	238
	1	59.0	8,450	4,343	3,211	300
	4	31.0	7,400	5,105	1,806	113
	8	31.0	16,800	14,112	1,949	75
	24	29.0	15,400	12,166	2,279	69
C. F.	0	16	5,800	2,657	2,668	381
	1	16	7,200	3,715	2,866	250
	4	15	9,650	5,616	3,203	288
	8	14	11,400	7,136	3,466	394
	24	20	6,600	3,168	2,600	413
	53	13	7,400	3,480	2,796	400
T. O.	0	17	9,900	6,276	2,990	134
	1	23	8,160	5,615	1,909	134
	4	21	10,050	7,919	1,729	75
	8	18	20,500	17,220	2,009	109
	24	10	14,950	12,528	1,973	150
J. E.	0	13.2	6,120	3,427	2,069	125
	1	18.0	5,700	3,146	2,143	106
	4	12.0	4,950	3,385	1,228	47
	8	28.0	14,550	12,455	1,484	32
	24	14.4	9,180	6,940	1,671	91

in steroids with a moderate drop in lymphocytes and eosinophils, and definite neutrophilia. These effects persisted, however, and were still present 24 hours after administration of the compound. In another subject (C. F.) no significant change was seen in either cells or steroids. In the other two subjects the changes were not marked, and in one (J. E.) were delayed.

TABLE V

Administration of 17-hydroxycorticosterone (Compound F) as the free alcohol, 100 mgm.

Subject	Mode of admin.	Time in hours	17-OH steroid levels, γ/100 ml. of plasma	WBC	PMN	Lymph	EOS
					Absolute number per mm.³		
J. B.	100 mgm. I. M. in 33% ethanol	0	16	4,400	1,892	2,112	113
		1	29	4,900	2,293	2,273	91
		4	13	6,700	3,953	2,386	31
		8	8	7,950	4,865	2,623	43
		24	11	5,550	2,620	2,542	119
O. H.	100 mgm. I. M. in 33% ethanol	0	18.0	7,800	3,947	2,902	300
		1	49.0	7,200	4,737	1,872	228
		4	21.0	16,400	15,055	1,016	12
		8	6.0	13,670	11,784	1,449	9
		24	2.0	8,280	4,951	2,600	241
J. B.	100 mgm. orally	0	3.0	6,400	2,650	3,366	75
		1	30.0	5,550	2,442	2,620	81
		4	34.0	8,700	6,177	2,123	6
		8	6.6	8,100	5,233	2,268	31
		24	10.6	6,400	3,213	2,777	81
O. H.	100 mgm. orally	0	5.0	8,150	4,304	2,950	300
		1	70.0	9,350	4,937	3,310	283
		4	22.5	20,100	17,889	1,688	13
		8	2.5	13,950	11,272	2,204	44
		24	9.0	7,250	3,814	2,624	256

TABLE VI

Oral administration of 17-hydroxy-11-dehydrocorticosterone (Compound E) as the free alcohol orally (200 mgm.)

Subject	Time in hours	17-OH steroid levels, γ/100 ml. of plasma	WBC	PMN	Lymph	EOS
				Absolute number per mm.³		
C. C.	0	8.0	7,850	4,698	2,685	294
	1	70.0	9,000	4,688	3,510	313
	4	14.0	6,600	4,963	1,439	38
	8	0.6	10,350	6,541	3,374	69
	24	7.0	10,900	6,300	4,164	450
J. C.	0	13.0	11,475	6,174	4,360	213
	1	58.0	9,550	5,635	3,476	213
	4	41.0	9,500	7,372	1,691	50
	8	7.5	12,300	8,561	3,247	25
	24	2.5	13,650	6,607	5,979	225
C. F.	0	5.4	9,250	4,126	3,811	519
	1	35.0	8,050	3,848	3,188	500
	4	41.8	7,200	4,939	1,814	175
	8	17.2	10,650	7,881	2,279	69
	24	14.5	9,150	5,398	2,965	325

Table V shows the effects of compound F given orally and intramuscularly (in ethanol solution) in the form of the free alcohol in a dose of 100 mgm. It may be seen that a rise in steroids with typical changes in cells occurred in each case by either route of administration with the same time relationships as are noted following oral F acetate ingestion. Oral administration of cortisone resulted in similar changes, as shown in Table VI.

TABLE VII

Administration of 200 mgm. 17-hydroxy-11-dehydrocorticosterone (Compound E) as the free alcohol, I. M.

Subject	Mode of admin.	Time in hours	17-OH steroid levels, γ/100 ml. of plasma	WBC	PMN	Lymph	EOS
					Absolute number per mm.³		
C. C.	Suspended in saline, I. M.	0	11.2	8,200	4,592	2,905	213
		1	5.6	7,650	4,192	2,693	206
		4	1.4	8,650	5,173	2,612	169
		8	5.6	10,600	6,508	3,456	175
		24	4.2	11,250	6,862	3,623	214
C. F.	Dissolved in 25% ethanol, I. M.	0	11.0	10,650	6,411	3,365	450
		1	38.0	11,700	8,237	2,481	406
		4	26.0	9,550	7,736	1,452	138
		8	21.0	14,800	11,574	2,723	75
		24	2.5	12,200	7,491	3,684	238
J. C.	Dissolved in 33% ethanol, I. M.	0	10.5	9,380	4,821	3,883	235
		1	46.0	9,050	5,321	3,168	238
		4	21.0	11,400	8,824	2,165	62
		8	21.6	16,125	10,933	4,676	59
		24	6.6	14,950	10,226	3,947	244

Table VII shows the effects of compound E given as the free alcohol in ethanol solution intramuscularly. Here again the rise in steroids with typical changes in cells was noted in each case. The time relationships are similar to those noted following oral administration of compound E as the free alcohol.

TABLE VIII

Administration of 17-hydroxy-11-desoxycorticosterone (Compound S) as the free alcohol or acetate

Subject	Dose and form of steroid and route of admin.	Time in hours	17-OH steroid levels, γ/100 ml. of plasma	WBC	PMN	Lymph	EOS
					Absolute number per mm.³		
O. H.	17-hydroxy-11-desoxy corticosterone acetate (Compound S acetate) 200 mgm. orally	0	18	9,550	4,737	3,839	244
		1	11	7,900	3,824	3,144	256
		4	9	7,900	4,172	2,924	288
		8	5	10,750	5,934	3,655	238
		24	11	6,700	3,510	2,640	306
O. H.	As above. Given I. M. in 10% ethanol	0	6	8,400	4,570	2,688	188
		1	15	7,350	4,292	2,264	238
		4	16	9,000	5,634	2,826	225
		8	10	10,000	5,880	3,220	200
		24	5	7,450	4,232	2,502	188
J. C.	17-hydroxy-11-desoxy-corticosterone (free alcohol), 200 mgm. orally	0	15	9,100	5,041	3,531	144
		1	14	9.100	4,787	3,786	144
		4	14	11,750	6,627	4,230	200
		8	11	13,250	7,253	5,088	400
		24	22	11,200	6,294	4,055	238
J. C.	Free alcohol of Compound S I. M., 200 mgm. in 25% ethanol	0	0.3	10,700	5,756	4,045	256
		1	9.6	10,950	6,745	3,504	200
		4	1.3	12,050	7,206	3,977	181
		8	1.3	15,900	7,905	7,028	325
		24	4.0	15,250	10,858	3,325	175

Intramuscular administration of free cortisone produced no changes when given suspended in saline, but when given in ethanol solution, typical responses occurred, as shown in Table VII.

Compound S, given as the acetate or free alcohol, either intramuscularly or orally, failed to affect significantly the blood steroid levels or the circulating leukocytes, as shown in Table VIII.

Table IX shows the diurnal variations in steroids and cells of two subjects, one of whom was studied on two separate days. Any changes which can be attributed to the administered hormone must fall outside this range.

TABLE IX

Diurnal variation

Subject	Time in hours	17-OH steroid levels, γ/100 ml. of plasma	WBC	PMN	Lymph	EOS
				Absolute number per mm.³		
O. H.	0 (8 a.m.)	10.0	8,250	4,901	2,656	150
	1	12.0	9,700	5,937	2,968	250
	4	11.0	8,500	5,525	2,397	331
	8	3.0	10,700	6,163	3,745	100
D. T.	0 (8 a.m.)	9.1	7,100	4,033	2,215	225
	1	7.5	7,900	5,087	2,244	175
	4	9.2	9,800	5,762	2,940	231
	8	8.1	9,400	5,189	3,272	269
O. H.	0 (8 a.m.)		7,450	4,038	2,563	256
	1		7,350	3,014	3,836	156
	4		9,200	5,484	2,889	206
	8		11,200	6,854	3,517	206

DISCUSSION

From these data it is apparent that following the oral administration of compound F or cortisone, either as the acetate ester or the free alcohol, there results a prompt rise in plasma 17-hydroxycorticosteroid levels, reaching a peak at approximately one hour and gradually falling thereafter. Although the highest levels appear to occur at one hour, no quantitative significance can be attached to the actual value since it is undoubtedly changing rapidly at this time. The levels at four hours are probably changing less rapidly and may be more indicative of the degree of change in steroid level which has occurred. This is well illustrated by the progressively rising four-hour levels in the individual who received various doses of compound F acetate. By eight hours in every case the steroids had returned to control levels, and in many instances they were below these values, suggesting that adrenal activity may have been suppressed by the previous phase of artifi-

cially increased steroid concentration. In some cases this was still apparent at 24 hours.

In contrast to the change in steroid levels, the changes in circulating leukocytes were rarely seen at one hour, and were usually maximal at four to eight hours after oral administration of the compounds. In many instances, when the maximal leukocyte alteration occurred at eight hours, the four-hour steroid level was relatively higher, suggesting that the maximum rise in steroids was later than one hour after administration of the steroid. Thus the leukocyte changes appear to lag behind the steroid change by about three to six hours. The occurrence of this lag is of considerable interest in relation to the mechanism of the cellular changes, but the reason for the delay is unknown at present.

Decreases in the average number of both eosinophils and lymphocytes were seen in every case when a significant elevation in steroid levels occurred. The alterations in these two cell types were, in general, closely parallel. At times, the numbers of each were higher at 24 hours than at any time previously. This eosinophilia and lymphocytosis may reflect a period of decreased adrenal activity as previously mentioned, or may be the result of increased cell production following their destruction (1). Changes in lymphocytes and eosinophils were well correlated with dosage in the individual who received graded doses of compound F acetate. However, the degree of cellular change from individual to individual could not be predicted from the steroid level. Thus, one individual may exhibit changes in the plasma steroid level of considerable magnitude and show only modest changes in the leukocytes, whereas another who may manifest a minimal elevation in the steroid level may show profound changes in the leukocytes.

Neutrophilia was frequently but not constantly seen in these subjects. It was not well correlated with changes in eosinophils or lymphocytes in time or degree. It seems likely that the neutrophilia was a result of hormone administration, although it does not appear to be a good measure of adrenal cortical activity. In terms of numbers of cells, however, the changes in neutrophils are by far the most prominent hematologic alteration, so that leukocytosis was noted frequently. The mechanism producing neutrophilia following steroid administration is entirely obscure; it should be pointed out that no increase in immature granulocytes occurred during the period of observation.

Although many of the subjects showed a decrease in monocytes at the same time that eosinopenia and lymphopenia were noted, this change in monocytes was difficult to evaluate since similar changes were noted in individuals observed throughout the day who did not receive steroids. No significant alterations in basophils were noted, nor were definite changes in volume of packed red cells or platelets seen in the few individuals in whom these measurements were followed.

Under the conditions of our experiments the intramuscular administration of compound F acetate did not produce significant changes in steroids or cells whereas such changes were seen following the intramuscular administration of the free alcohol. This phenomenon may be due to the fact that the free compound was either dissolved or suspended in ethanol, whereas the acetate was in a saline-suspending medium. The fact that the suspending medium is of importance in absorption of steroids from intramuscular sites is demonstrated by the observation that free cortisone produced good effects when administered in ethanol, but not when administered in saline. It is also possible that the presence of the acetate group in the compound results in delayed absorption and utilization. Further studies are under way to elucidate this problem of absorption and hydrolysis of the ester. It should be emphasized that these studies of the usual steroid acetate suspensions for intramuscular injection were carried out by the use of single large doses and that the effect of repeated administration was not tested. It is quite possible that repeated administration might alter the steroid levels as well as cellular relationships. On the other hand, the relatively slow clinical effect of intramuscular cortisone acetate in contrast to its oral administration is no doubt related to the pharmacological implications of these studies.

Compound S given as the acetate or free alcohol was ineffective in producing changes in either steroid levels or cells, by either route of administration. Since this compound was prepared in a different physical form from the compounds E and F, it is possible that differences in particle size

may play a role in its failure to increase steroid levels.

SUMMARY AND CONCLUSIONS

1. The administration by mouth of cortisone, cortisone acetate, compound F (Kendall) and compound F acetate to normal human subjects regularly resulted in an elevation of blood levels of 17-hydroxycorticosteroids, and in neutrophilia, lymphopenia, and eosinopenia. The administration of cortisone and compound F intramuscularly in alcoholic solution produced similar responses. The administration of compound F acetate intramuscularly did not result in these changes, whereas the administration of cortisone acetate produced them irregularly. No significant changes followed the administration of compound S or compound S acetate either orally or intramuscularly.

2. The maximum elevation of blood 17-hydroxycorticosteroid levels occurred approximately one hour after administration of the compound by either route. The maximum change in blood cells took place four to eight hours after administration.

3. Changes in eosinophils and in lymphocytes following steroid administration appeared to be closely related to each other, whereas changes in neutrophils, although generally greater in magnitude, appeared not to be closely related to other cellular changes either in time or degree.

4. The cellular changes that occurred following administration of these steroids appeared to be closely related to dosage and plasma 17-hydroxycorticosteroid levels.

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INTERRELATIONS OF MAGNESIUM, POTASSIUM, PHOSPHORUS, AND CREATINE IN SKELETAL MUSCLE OF MAN [1]

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In latter years the growth of methods has led to a detailed understanding of the concentration and distribution patterns of electrolytes and water in the extracellular liquids. More recently attention has been directed toward the intracellular phase. The greater bulk of observations has been concerned with the most abundant intracellular cation, potassium. Much less is known about magnesium, second only to potassium with respect to concentrations within cells.

There are many indications of the intimate role which magnesium plays in modulating neural excitability and muscular contraction (1–7); in catalysing several enzymic processes concerned with the transfer, storage and utilization of energy (8–14); and, perhaps, in the adjustment of overall bodily economy reflected in temperature regulation and hibernation (15, 16). Yet little is known of the patterns of its ebb and flow into and out of cells, or, indeed, of the concentrations obtaining within cells during most diseased states. This study was begun in order to discover what changes, if any, occurred in the magnesium content of skeletal muscle during certain clinical states marked by disturbances of electrolyte metabolism and accompanied occasionally by asthenia.

For purposes of reference, concentrations of magnesium and of potassium in muscle were measured at the same time. It soon became apparent that even in the most diverse states of disease, as well as in health, an extraordinarily fixed relationship obtained in muscle between concentrations of magnesium and of potassium: the two major cations had entered or left the muscle cell in such amounts as to maintain a constant relation one to the other. This observation suggested further analysis of the relation held by these cations, potassium and magnesium, to representative intracellular anions. Phosphorus and creatine were selected for study because of their abundance, their relation to each other as creatine phosphate and to potassium in phosphorylating reactions (17–19), and because of observations which have suggested that potassium and phosphorus cross cell barriers in a relatively fixed proportion (20–22).

MATERIAL AND METHODS

All samples of muscle for analysis were obtained by biopsy. The normal series was secured from 15 patients undergoing surgical procedures under general anesthesia for conditions not associated with clinical or chemical evidence of disturbed electrolyte metabolism. The remaining 15 samples were obtained by surgical excision under local procaine anesthesia of overlying tissues; no anesthetic solution was introduced below the overlying muscle sheath. Five such samples were taken from muscles which had undergone extensive *atrophy*. Another five samples were excised from the gastrocnemii of patients who, because of a variety of diseases, had developed *hypokalemia*. The final five samples of muscle came from patients who had developed *hyperkalemia* in association with severe renal failure owing to several causes. Because the underlying disease process was complex in each instance, the patients were divided arbitrarily into these two groups on the basis of a predominant pattern. For example, patients L. W. and A. M. were assigned to the hypokalemic group because the concentrations of potassium in serum were at the lower limit of the normal range.

The samples of muscle were excised, quickly blotted to remove superficial blood, and trimmed of any obvious tendinous or fascial shreds. A representative portion of each sample from the abnormal groups was separated at once and fixed for microscopic study. The muscle was then frozen rapidly by introducing it into a glass container packed in dry ice. Subsequently, the muscle was split into fragments which were weighed in the frozen

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TABLE I

Nitrogen content of muscle fat

Gm. fat per 100 gm. fresh muscle	Gm. N per 100 gm. extracted fat	% error in total NCN
4.4	.4	.7
9.5	.5	2.2
10.4	.5	2.0
14.1	.5	2.5
20.0	.3	2.9
35.1	.6	8.9
74.8	.5	15.2

state in a refrigerated balance. Duplicate, usually triplicate, and occasionally quadruplicate fragments, weighing between 0.5 and 1.0 gm., were homogenized in glass-distilled water by a Lucite pestle and tube modified from Potter's design (23). The basic system of analysis of the aqueous homogenates followed that previously described (24). Creatine was determined in the alkali digest as creatinine by the method of Peters (25). Fat content was estimated by extracting the aqueous homogenate with ethyl ether and weighing the dry residue. The fat was then analyzed for its nitrogen content. With only a few exceptions, chemical analyses were performed in triplicate on each homogenate made from each of three or four fragments of a single sample of muscle.

The continuing problem of selecting a base to which to refer the concentrations of intracellular constituents has been discussed before (24). Experimentally induced muscle atrophy has been studied in rabbits (26); and with atrophy, that fraction of total muscle protein provided by collagen became proportionally larger as intrafibral protein was lost. Such a dilution of true muscle by fibrous or other non-muscular tissue in certain diseased states led to the adoption of non-collagenous nitrogen (NCN) as a reference base in order to set one parameter in terms of an intracellular substance rather than a mixed anatomical mass of muscle cells and extra-

TABLE II

SERUM CONCENTRATION

Subject	Sex	Age	Diagnosis	Muscle	Atrophy (microscopic)	Weakness	K mEq /l.	Mg mEq /l.	P mg %	Na mEq /l.	Cl mEq /l.	Ca mg %	CO_2 mEq /l.	NPN mg %	T.P. g %
ATROPHY															
F.L.	f	84	Severe peripheral arteriosclerosis Hip fracture	G M			4.5	1.8	4.4		96.8		23.3	44	5.9
H.S.	m	48	Severe peripheral arteriosclerosis	BF	+++		4.0	1.8	5.8						
J.B.	m	25	Anterior poliomyelitis	G	+		4.5	2.0						39	
J.M.	f	30	Alcoholic polyneuritis	G	++		4.5	1.6						28	
E.A.	m	69	Amyotrophic lateral sclerosis	TB	+		4.9	2.0	3.6					26	
HYPOKALEMIA															
L.W.	f	48	Chronic pyelonephritis	G	0	++	3.5	1.0	4.7	98	76.5	7.8	14.5	93	5.9
V.C.	f	23	Ulcerative colitis	G	0	++	2.7	2.1	2.7	131	94.1	9.0	20.0	29	6.3
C.H.	f	34	Lymphosarcomatosis (with protracted emesis)	G	0	+++	2.5	1.5	2.4	131	85.8	20.8	39.0	50	5.6
A.M.	f	63	Adenocarcinoma of esophagus with metastases to bone	G	++	++	3.7	1.6	2.8	138	104	17.1	30.0	40	7.3
M.H.	f	46	Hypertensive cardiovascular renal disease; salt losing syndrome	G	0	++	4.4[a]	3.3	7.1	115	79.0	7.5	17.4	104	4.9
HYPERKALEMIA															
L.H.	m	60	Hypertensive cardiovascular renal disease	G	0	0	6.4	2.5	5.2	125	104	6.2	21.0	121	5.9
P.C.	f	24	Lupus erythematosus renal involvement	G	0	+	7.3	2.3	4.8	144	106	8.5	20.0	128	4.8
I.N.	m	65	Chronic pyelonephritis	PM	0[b]	0	5.2[c]	1.6	8.9	127	98	8.1	9.4	122	7.0
R.S.	m	55	Polycystic kidneys	PM	0	0	5.7	2.2	6.6	144	109	10.2	21.2	125	5.9
J.G.	f	61	Hypertensive cardiovascular renal disease; salt losing syndrome	RA		++	6.2	2.5	3.0	145[d]	94.3	8.8	23.2	130	6.9

G - Gastrocnemius
PM - Pectoralis major
BF - Biceps femoris
TB - Triceps brachii
GM - Gluteus medius
RA - Rectus abdominis

a. Potassium values in this patient had ranged from 2.5 to 3.1 mEq/l. during the week prior to biopsy. Value obtained the day before was 3.1 mEq/l.
b. Grossly atrophic muscle mass. No microscopic evidence of atrophy in small fragment sectioned.
c. Potassium values in this patient had ranged from 6.0 to 6.3 mEq/l. during the week prior to biopsy. Value obtained the day before was 6.2 mEq/l.
d. Sodium values in this patient had ranged from 126 to 135 mEq/l. during the two weeks prior to biopsy.

TABLE III

MUSCLE CONCENTRATION

SUBJECT	NCN	Mg	K	P	Creatine	Mg	K	P	Creatine
		per kilogram of wet weight				per gram of non-collagenous N_2			
NORMALS	g.	mEq.	mEq.	g.	g.	mEq.	mEq.	g.	g.
N.D.	23.1	15.7	89.7			0.68	3.9		
G.H.	28.0	16.5	92.0			.59	3.3		
A.K.	29.4	20.6	112.5			.70	3.7		
C.W.	26.0	16.2	93.8			.62	3.6		
E.H.	*	*	*			.62	3.5		
N.L.	*	*	*			.57	3.3		
L.H.	24.2	14.4	82.2			.60	3.4		
J.B.	24.7	13.4	77.0			.55	3.1		
B.I.	26.1	15.9	90.9			.61	3.5		
P.L.	28.5	15.6	94.1			.55	3.3		
W.S.	29.9	20.0	111.6	2.08	3.90	.67	3.7	0.071	0.130
W.B.	28.3	14.6	86.1	1.62	2.96	.52	3.1	.058	.105
L.B.	29.5	18.1	107.9	1.96	4.46	.61	3.7	.068	.152
E.N.	28.6	18.0	106.9	1.89	4.90	.63	3.7	.067	.171
M.J.	25.8		91.9	1.58	3.38		3.6	.062	.132
Mean :	27.0	16.4	94.6	1.83	3.91	0.61	3.5	0.065	0.138
S.D. :	2.5	2.1	11.7	0.22		0.06	0.3	0.005	
ATROPHY									
F.L.	20.8		78.8	1.31	2.80		3.8	0.063	0.134
H.S.	20.5	11.6	63.3			0.57	3.1		
J.B.	23.8	15.5	85.4			.66	3.6		
J.M.	21.4	13.5	72.9			.63	3.4		
E.A.	29.9	18.9	112.1			.63	3.8		
Mean :	23.3	14.8	82.3			0.62	3.5		
HYPOKALEMIC									
L.W.	22.7		74.6	1.36	2.47		3.3	0.060	0.109
V.C.	29.6	15.6	81.3	1.75	3.65	0.53	2.7	.060	.123
C.H.	25.6	12.2	75.8	1.40	3.15	.48	3.0	.055	.123
A.M.	17.2	8.7	48.2	1.06	1.59	.50	2.8	.061	.092
M.H.	24.3	14.4	76.5	1.45	2.64	.60	3.2	.060	.108
Mean :	23.9	12.5	71.3	1.40	2.70	0.53	3.0	0.059	0.111
HYPERKALEMIC									
L.H.	20.9	17.6	95.7	1.46	2.85	0.84	4.6	0.070	0.137
P.C.	21.9		84.9	1.44	3.45		3.9	.065	.158
I.N.	21.4	15.7	82.7	1.53	2.11	.70	3.9	.070	.098
R.S.	29.1	19.1	115.8	1.91	4.23	.66	4.0	.066	.146
J.G.	20.1	10.6	62.5	1.15	2.23	.53	3.1	.057	.112
Mean :	22.7	15.8	88.3	1.50	2.97	0.68	3.9	0.066	0.130

* Owing to an error in dilution, only the ratios of electrolyte to NCN were obtained.

fibral tissues. Support for the validity of this analysis appears below in the study of atrophied muscle where the apparent depletion of magnesium, potassium and phosphorus (referred to wet weight) vanished when these same elements were referred to an intracellular substance, NCN, an index of intrafibral protein (Table III).

The question of the size of the contribution to the total NCN by the nitrogen contained in fat was examined. As demonstrated in Table I, the nitrogen contributed by fat, but appearing as NCN, becomes significant only when the fat content of the muscle sample exceeds one-fifth. The NCN values for all muscle samples of the hypokalemic and hyperkalemic series were corrected for NCN contributed by fat. In only two specimens of muscle did the fat content of the muscle exceed 20 per cent.

The values determined, which are expressed to imply that they represent intracellular concentrations, have not been corrected for the contribution of these substances from the intravascular and interstitial spaces. Because of the high concentration gradients between the intra- and extracellular phases, it may be calculated that the error introduced by failure to apply this correction does not exceed 2 per cent.

RESULTS

The clinical material and the concentrations of the various constituents in serum on or before the day of biopsy are presented in Table II. In these laboratories the normal range for serum magnesium is 1.7 to 2.2 mEq./l. and for serum potassium 3.5 to 5.0 mEq./l. The concentrations found in the samples of muscle appear in Table III. The values for normal muscles were of the same order as those calculated on a fat-free basis by Shohl from the literature (27). The ionic concentrations in atrophied muscle, when referred to NCN, were the same as those discovered in normal muscle, an inevitable result of the proportional depletion both of ions and of contractile proteins.

When the concentrations of potassium and of magnesium were plotted as a ratio, this ratio appeared to be constant and equal for all samples studied, whether from normal subjects, atrophied muscle, or from patients with frank disturbances of electrolyte metabolism (Figure 1). In this figure each point represents the mean value of two or three fragments from one sample of muscle. The average variation from its mean within each group of fragments was less than 5 per cent. The regression curve intercepts the origin.

A similar fixity of the concentration ratios for K/P (55.1, S.D. = ± 4.6) and for Mg/P (9.5, S.D. = ± 0.8) was found (Figure 2). However, a linear extrapolation of these regression curves intercepts the abscissa at a value corresponding to approximately one-third of the content of total potassium in normal muscle (Figure 3).

The relations of the concentrations of potassium (and therefore of magnesium) to those of creatine

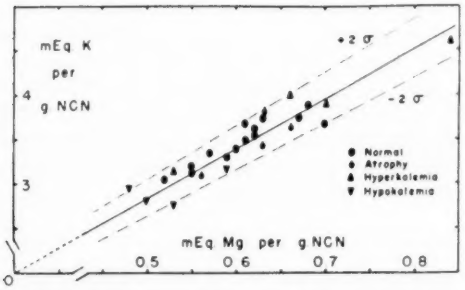

FIG. 1. RELATIONSHIP BETWEEN K AND MG IN 26 MUSCLE BIOPSIES

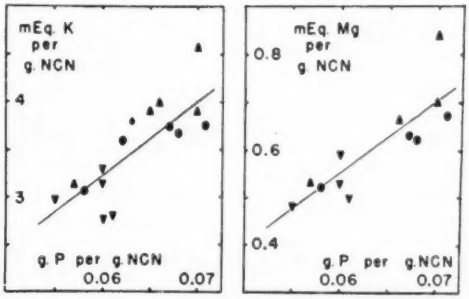

FIG. 2. RELATIONSHIP BETWEEN K AND P, AND MG AND P IN MUSCLE BIOPSIES

were less clear (Figure 4). Nevertheless, if the samples are divided arbitrarily into two groups on the basis of their having contained more or less than 3.5 mEq. K per gm. NCN, the mean normal concentration, then there was a statistically significant reduction in creatine content in those muscles with diminished amounts of cation.

The wide variations in the ratios of potassium to magnesium obtaining in serum, in sharp contrast to the small variation in muscle, are presented in Figure 5. A three-fold increase in the K/Mg ratio in serum was accompanied by no significant change in the ratios of muscle. The same constancy of the K/P and Mg/P ratios in muscle were found despite a five-fold variation of these same

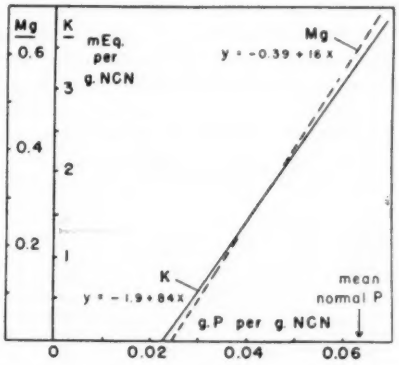

FIG. 3. REGRESSION CURVE CALCULATED FROM DATA OF FIG. 2

When concentration of K is zero, P = 0.023 gm. per gm. NCN. Normal P concentration = 0.063 gm. per gm. NCN.

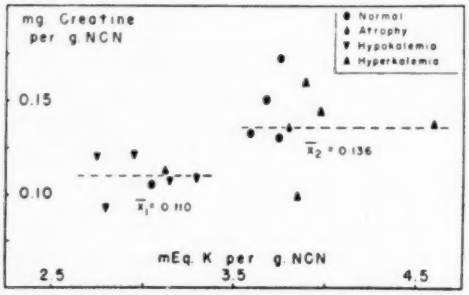

FIG. 4. RELATIONSHIP OF CREATINE AND K IN MUSCLE

The mean creatine of the "low" K group is 0.110, of the "high" K group is 0.136 gm. The probability, calculated from the "t" test, that this difference occurred by chance is less than one in 50. "t" = 2.82; $P < 0.02$.

ratios in serum. In this limited series, a significant depletion of muscle K accompanied hypokalemia in two of five instances; and at least two of the five patients with hyperkalemia were found to have appreciable intracellular accumulations of potassium. Because of the coupled relation of the major cations, the magnesium contents of muscle reflected those of muscle potassium but showed no clear relation to the concentrations of magnesium in serum.

The ratios of serum potassium (mEq./l.) to muscle potassium (mEq./gm. NCN) average about 1.3 in normal subjects. In the hypokalemic group the average ratio was 1.1 (0.8–1.3); and in the hyperkalemic group it was 1.6 (1.3–2.0). The small groups reported here do not lend themselves to valid analysis, but it is of some interest to note that in each group, that patient with the most divergent ratio also exhibited the greatest degree of muscular weakness as assessed by relatively crude clinical measurements.

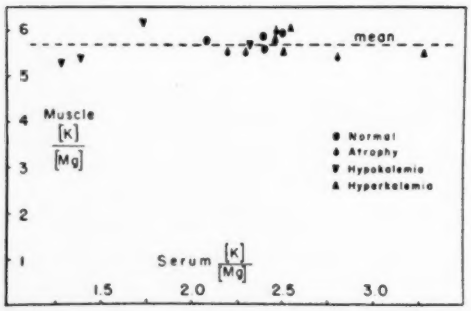

FIG. 5. INDEPENDENCE OF MUSCLE RATIOS AND SERUM RATIOS OF K TO MG

The constancy in muscle of the ratios K/Mg, K/P and Mg/P might suggest that these relations followed necessarily the simple reduction or accumulation of muscle tissue containing a normal protoplasmic pattern, and thus no differential loss or gain of any intracellular constituents had occurred. That this is not likely to have been the case is indicated in Figure 6, where it may be seen that the relation of potassium (and, therefore, of Mg and P) to NCN was random. In muscles ranging in content of NCN from 20 to 30 gm. per kgm. wet weight, the potassium content was found

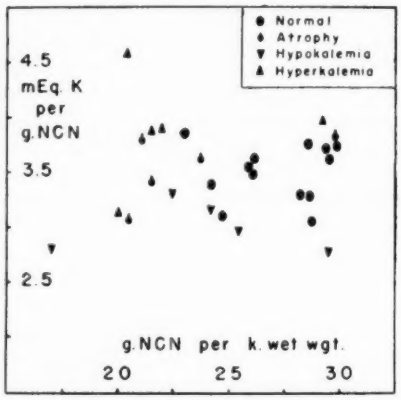

FIG. 6. RANDOM RELATION OF K PER MGM. NCN TO NCN PER KGM. OF WET WEIGHT

to vary through a range of 1.1 mEq. per gm. NCN, or approximately one-third of the mean total potassium content. The most striking example was seen in patient L. H. in whose gastrocnemius the NCN was reduced to 20.9 gm. per kgm. wet weight, while K had accumulated to a concentration of 4.6 mEq. per gm. NCN (Table III). This variation indicates that the changes discovered were not the result simply of dissolution or expansion of normal intracellular mass.

DISCUSSION

Direct determinations of intracellular magnesium, potassium and phosphorus in human skeletal muscle have demonstrated an extraordinarily fixed

relationship of these substances in the face of wide distortions in the pattern of the extracellular milieu. This observation was unexpected, for there are several reports indicating that in the muscles of rat and frog, magnesium, potassium, and phosphorus may rise and fall in reciprocal or even dissociated fashion (28–31). However, other studies are consonant in whole or in part with the relation of these intracellular constituents which has been described here. For example, potassium is known to move with inorganic phosphate into the cell in conjunction with the phosphorylation of glucose (21, 32). A linked transfer of this same kind has been seen in the simultaneous fall in serum concentration of potassium and of inorganic phosphorus following the intravenous infusion of glucose, fructose, and epinephrine (33), and following the injection of insulin in dogs (20). In the latter case, following insulin, there was also a slight but consistent fall in serum magnesium. Recently Eliel, Hellman, Pearson, and Katz (22) have demonstrated a simultaneous loss of intracellular potassium and phosphorus from skeletal muscles of patients under treatment with adrenocorticotropic hormone or cortisone.

Evidence supporting the premise that magnesium is also closely related in the net flux of intracellular substances, has been more indirect. Fenn and Haege's study of frog muscle showed that magnesium might move in the same or opposite direction with respect to potassium, depending on the kind of external or internal stress which was applied (29). Furthermore, from balance studies during the onset and treatment of diabetic acidosis, it was estimated that several cations were wasted or saved in proportion to the fraction of intracellular base which they formed (34, 35). As a consequence Butler has emphasized the necessity for a simultaneous repair of the deficit of intracellular potassium, magnesium and phosphorus in diabetic acidosis (36). Additional support for this view is implicit in the observation that the serum concentrations of all three substances fall simultaneously following the initial therapy of diabetic acidosis with insulin and NaCl, and the presumed transfer of intracellular constituents from the extracellular fluids into cells (37).

Mangun, Reichle and Myers reported a decrease in potassium, phosphorus and creatine content of skeletal muscle in patients who had died in congestive heart failure (38). This deficiency they interpreted to be the net effect of a simultaneous escape of these three substances from the "cells" following the breakdown of a di-potassium salt of phosphocreatine. Such an explanation would appear to be inadequate now to explain the relation of potassium, phosphorus and creatine reported here. If the linear extrapolation of the cation-phosphorus ratio in Figure 3 be valid, some 60 per cent of total phosphorus is related to cell cation. However, not more than 20 per cent of total phosphorus is known to be present in the form of phosphocreatine (39). The difference, 40 per cent of the total, then, represents the approximate amount of phosphorus related to cell cation but not associated with creatine. It is unlikely, therefore, that the association of the flux of potassium, magnesium and phosphorus described in this report is more than a partial function of phosphocreatine synthesis and hydrolysis. The observation that patients with supernormal accumulations of intracellular potassium did not develop comparably elevated creatine concentrations is additional evidence against the inference that potassium accumulates in the cells only as a result of the synthesis of phosphocreatine.

One possible interpretation of the association of an intracellular potassium deficit with a deficit of creatine is that normal synthesis of phosphocreatine requires an adequate concentration of intracellular electrolyte. Phosphocreatine synthesis in this case would be dependent on muscle electrolyte content, rather that the reverse. Such a relation is consonant with certain recent knowledge of creatine metabolism. Several investigators have described the essential role of potassium in phosphorylation of creatine *in vitro* (17–19). On the other hand, it is probable that supernormal accumulations of potassium are not accompanied by comparable storage of creatine because the muscle has become saturated with respect to creatine, which, as has been shown for the rat, cannot be stored in amounts greatly exceeding that which occurs normally (40, 41).

The justification for using serum magnesium, potassium and phosphorus concentration ratios as indices of comparable interstitial fluid ratios may be questioned on the grounds that the degree

of binding of these substances may vary. Previous reports have indicated, however, that serum potassium exists in a state in which its distribution between serum and interstitial fluid conforms to the values predicted by the Gibbs-Donnan equilibrium (42, 43). Serum phosphorus has long been known to exist in a similar state, the fraction bound to serum protein being small (44). Although high proportions of bound to total magnesium in serum have been reported to occur in hyperthyroidism (45), in most other states the proportion is sufficiently stable so that it is likely that the wide range in concentration in total magnesium in sera reported here was reflected at least approximately by a similar wide range in concentration in magnesium in interstitial fluid. On the assumption, then, that concentration ratios of serum electrolyte ratios provide approximate indices of similar interstitial fluid proportions, the conclusion has been drawn that gross distortions in composition of extracellular fluid and wide variations in electrolyte concentration gradients need not be accompanied by any change in the proportionality of potassium, magnesium, and phosphorus in the cell. Attention may be drawn to the fact that by no means all examples of intracellular electrolyte distortions have been studied. The possibility remains, therefore, that the fixity of intracellular concentration ratios might fail *1*) in the course of abrupt shifts of an acute nature or *2*) in the face of other diseases not examined.

It would appear, nevertheless, that whatever may be the mechanism responsible for the maintenance of fixed proportions of these electrolytes in muscle, it continues to operate effectively during certain states of depletion or of accumulation, and to defend these constant relationships during extensive alterations produced in interstitial liquids by impaired function of kidney and bowel. This conclusion implies that attempts to repair intracellular deficit of potassium and phosphorus might be accompanied reasonably by simultaneous repletion of magnesium, and perhaps several other constituents of muscle cells.

With respect to possible mechanisms concerned in the guarding of concentration ratios of intracellular electrolytes there are no data reported here which resolve substantially the differences to be found in current concepts. These interpretations vary from those which imply that the retention or exclusion of ions is the result wholly of active "pump"-like mechanisms to those which suggest that pure physicochemical forces such as charge, density, and spatial arrangement of the intracellular proteins and ions, are alone responsible for the gradients which occur (46). The interrelations which have been described in this report are compatible with either mechanism or, perhaps, with both.

Initially, these studies were undertaken in the hope that there might be disclosed some coordinated relation between the pattern of intracellular electrolytes and the occurrence of muscular weakness. There are only vague suggestions in these limited data that an excessive change in the extracellular-intracellular concentration ratio of potassium, at least, may be associated with weakness. More extensive measurements may in time establish or exclude what now appears to be only a hint, made especially tenuous by the fact that these patients were suffering from protracted illnesses accompanied variously by anemia, inanition, azotemia and multiple disturbances of electrolyte metabolism.

SUMMARY

1. In normal muscle the mean values for potassium, magnesium and phosphorus and creatine were, respectively, 3.5 mEq., 0.61 mEq., 0.065 gm., and 0.138 gm., referred to one gm. of noncollagenous nitrogen (NCN).

2. In atrophied muscles the cation concentrations were reduced in terms of wet weight but, when referred to NCN, were identical with the normal series.

3. The mean ratio of potassium to magnesium (referred to NCN) was 5.67, with a standard deviation of but 0.18, for *all* muscles studied, whether normal, atrophied, or from patients with sweeping electrolyte distortions in serum leading to accumulation or loss of cations.

4. A similar, but not identical, constant relationship of potassium and of magnesium to total phosphorus was observed.

5. The mean creatine concentration in muscles containing less than 3.5 mEq. K per gm. NCN was significantly less than in those muscles with higher concentrations of potassium.

6. Constant intracellular proportions of potassium, magnesium and phosphorus were observed

despite wide variations in the proportions of these substances in the serum.

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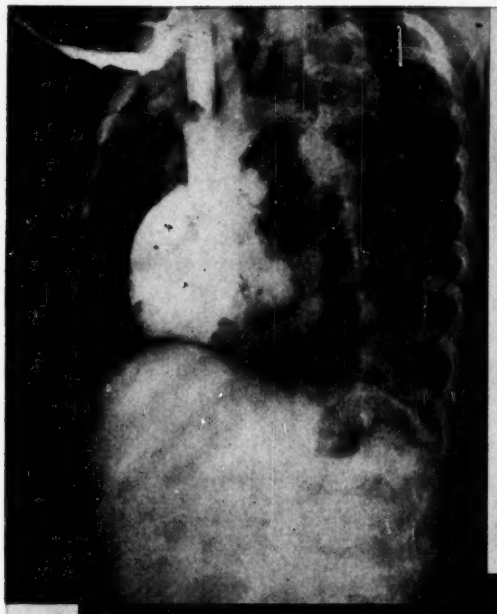

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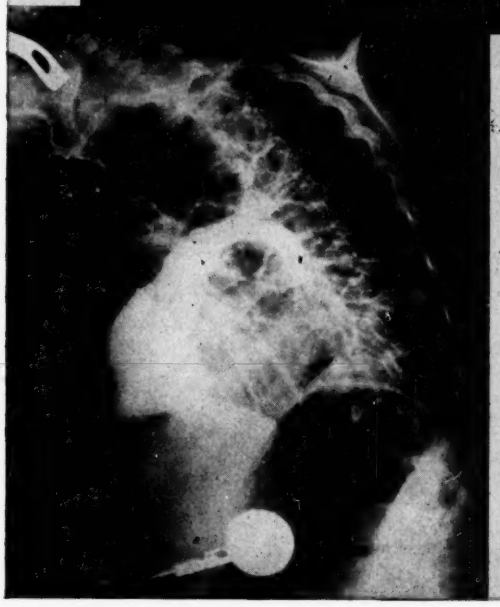

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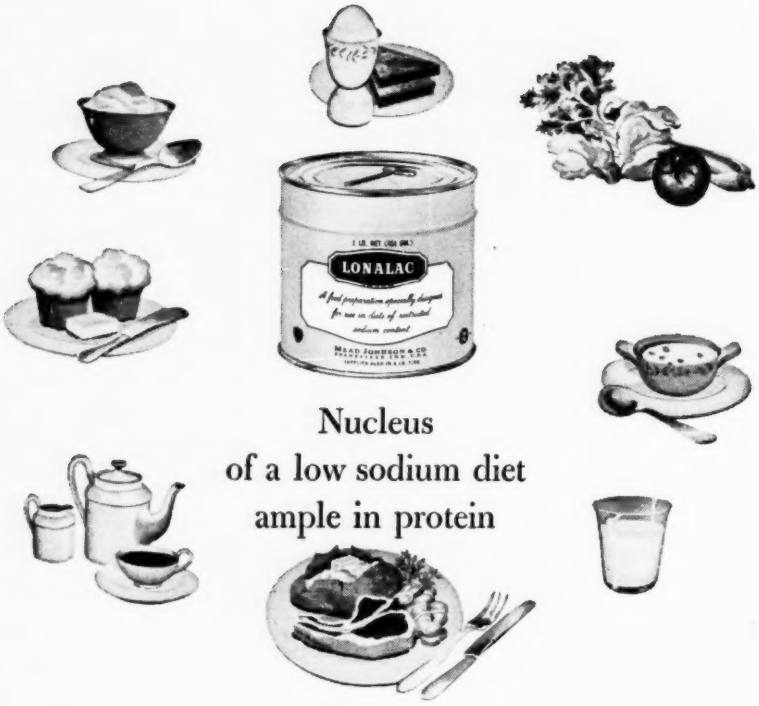

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